

# Stem Cell And Tissue Engineering: Baics

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# Definition of stem cells

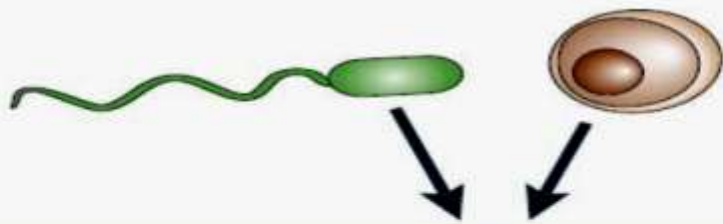
- Unspecialized cells
- Have ability to Self-Renewal
- Have ability to Differentiate to specialized cells

# Classification

1. According to potency
2. According to source

# According to potency

- Totipotent
- Pluripotent
- Multipotent
- unipotent



**Totipotent**



Zygote



**Pluripotent**



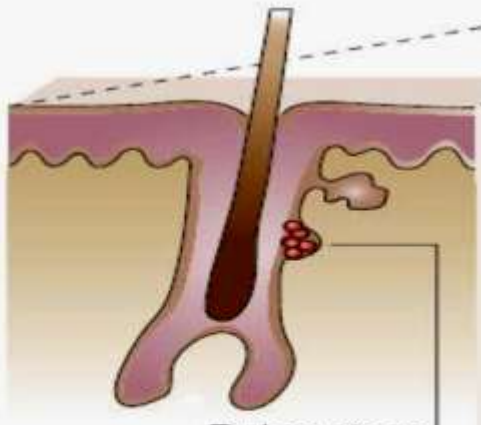
Embryonic stem cells



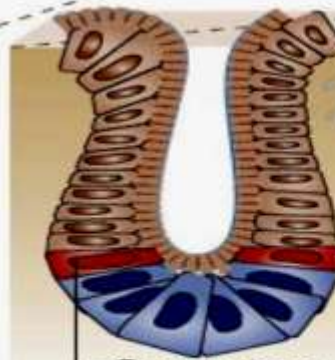
ICM  
EE

Blastocyst

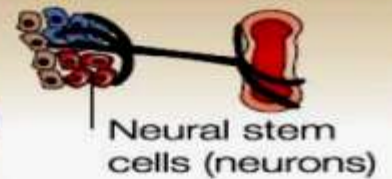
**Multipotent**



Bulge stem  
cells (skin/hair)



Crypt stem  
cells (gut)



Neural stem  
cells (neurons)



Haematopoietic  
stem cells (blood)

# According to source

- Embryonic stem cells
- Fetal stem cells
- Umbilical cord stem cells
- Adult stem cells

# Embryonic stem cells

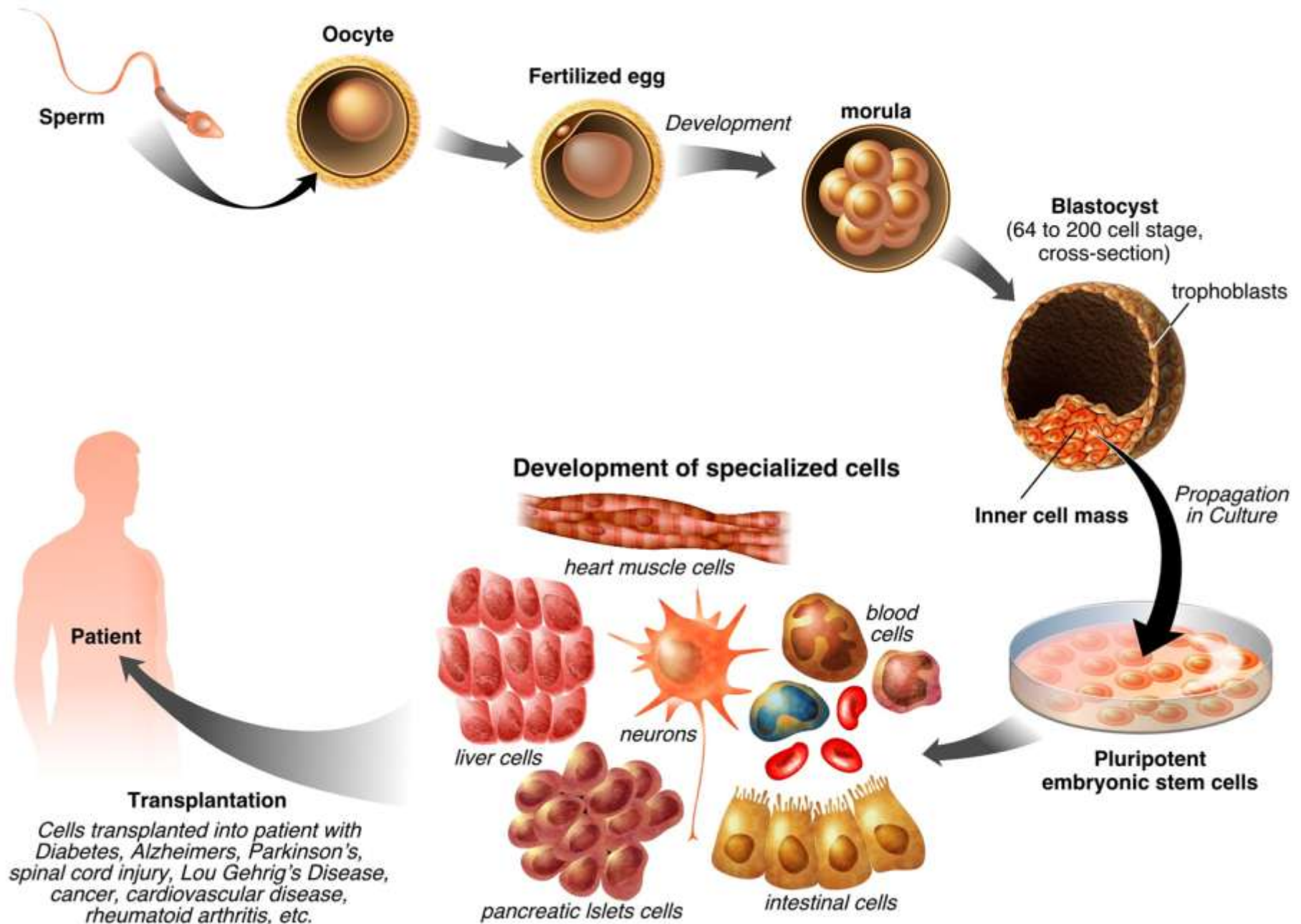
# How ESCs are obtained?

1. Excess fertilized eggs from IVF (in-vitro fertilization) clinics
2. Embryos made exclusively in order to receive ESC
3. Therapeutic cloning (somatic cell nuclear transfer/ SCNT)

# FERTILIZATION



# Stem Cell Therapy





Blastocyst.

Courtesy of Wellcome Library, London.

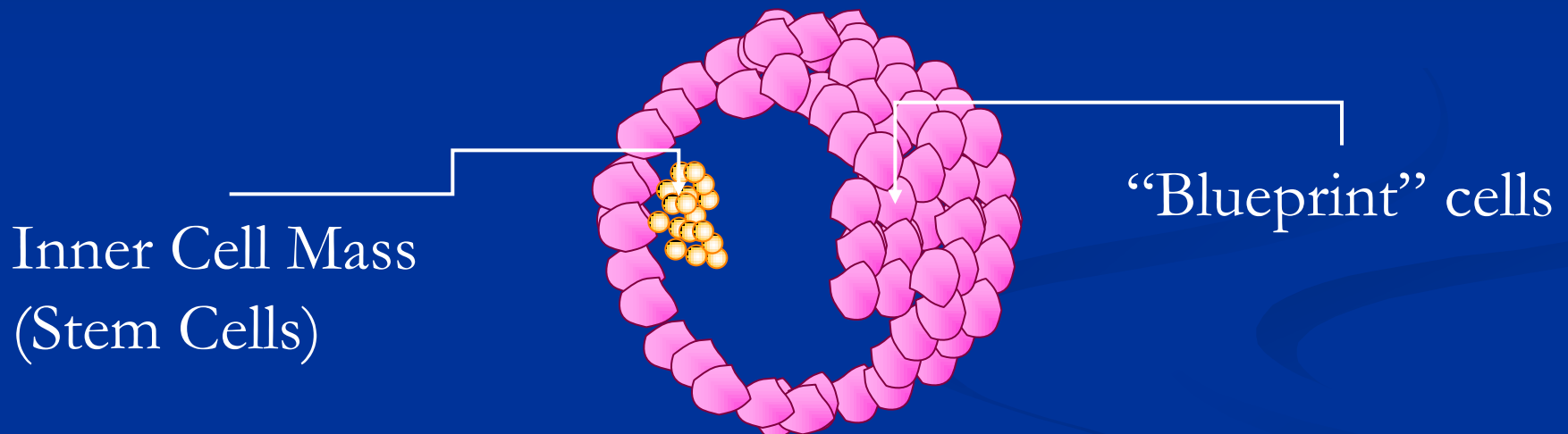


Blastocyst opened to reveal the inner cell mass.

Courtesy of Wellcome Library, London.

# A primer on Human Embryonic Stem Cells

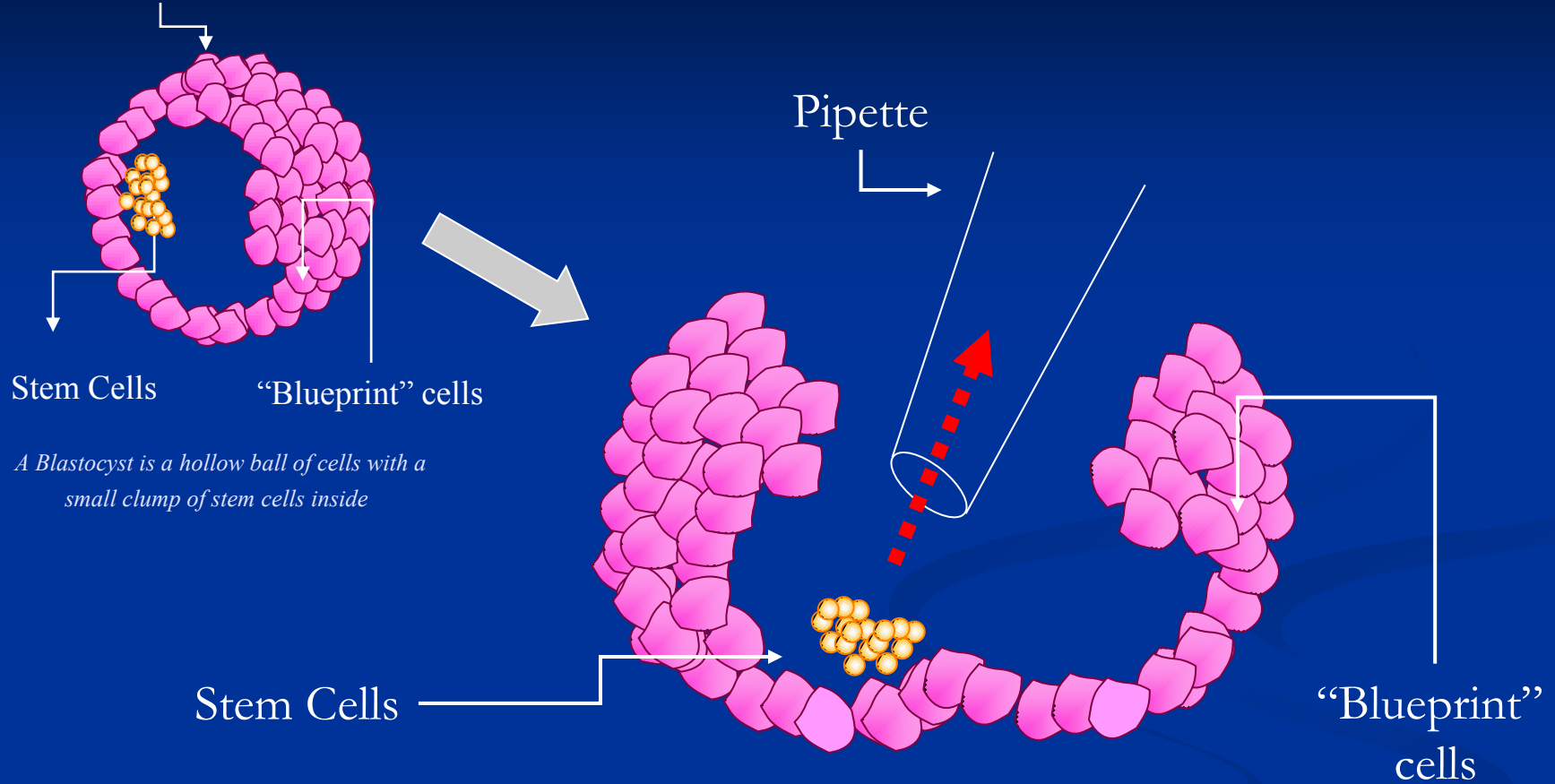
Blastocyst -  
from In Vitro Fertilization Clinic



A Blastocyst is a hollow ball of cells with a small clump of stem cells inside

# Human Embryonic Stem Cells

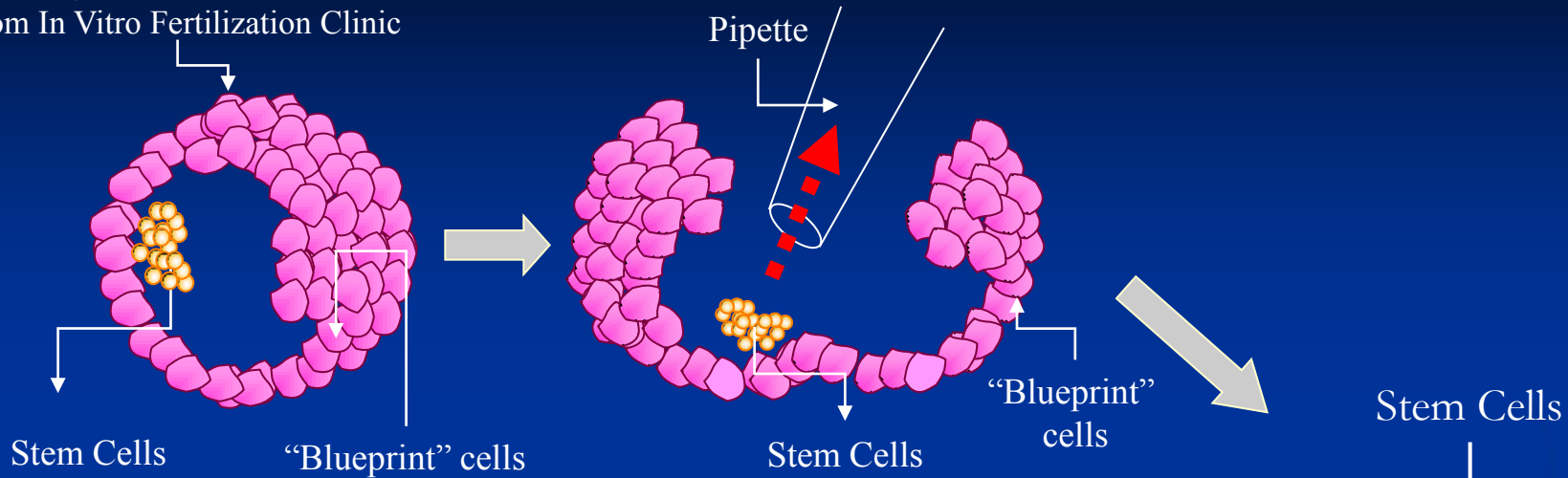
Blastocyst -  
from In Vitro Fertilization Clinic



To remove the stem cells, the Blastocyst is opened and the stem cells removed with a pipette

# Human Embryonic Stem Cells

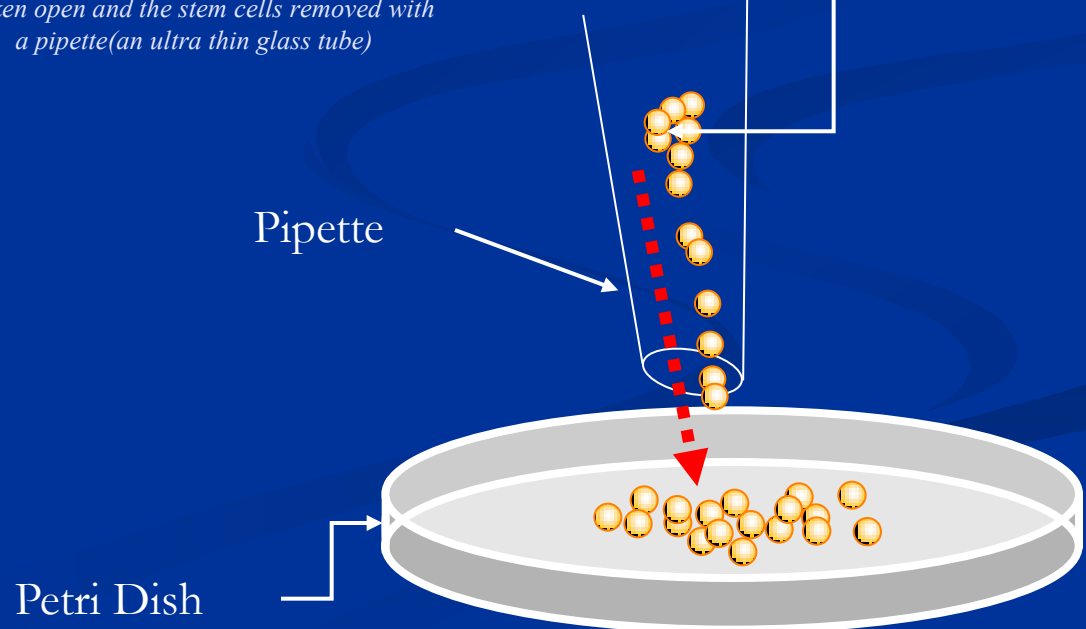
Blastocyst -  
from In Vitro Fertilization Clinic



*A Blastocyst is a hollow ball of cells with a small clump of stem cells inside*

*To remove the stem cells, the Blastocyst is broken open and the stem cells removed with a pipette(an ultra thin glass tube)*

The stem cells are  
placed in a  
dish and are fed and  
cared for  
(each blastocyst =  
1 stem cell line)

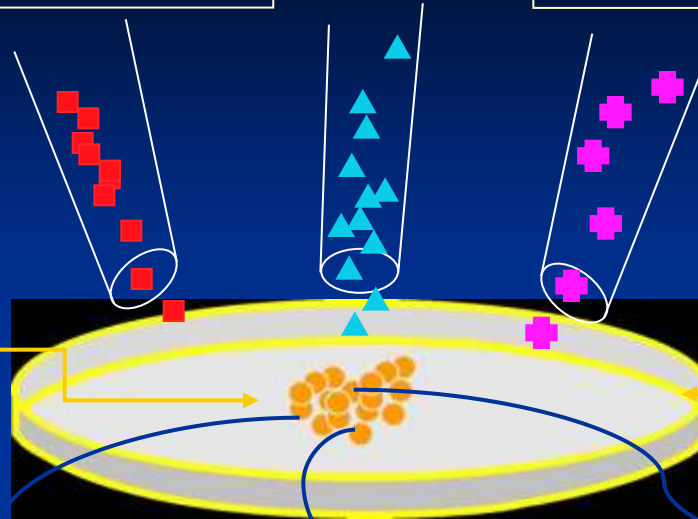


**Growth factors**

**Chemical cues**

Stem Cells

Petri Dish



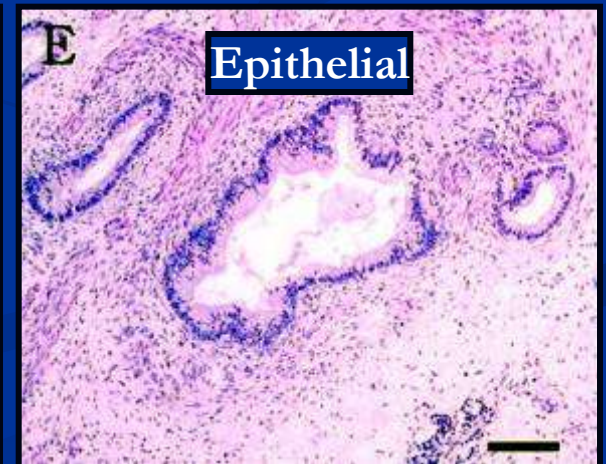
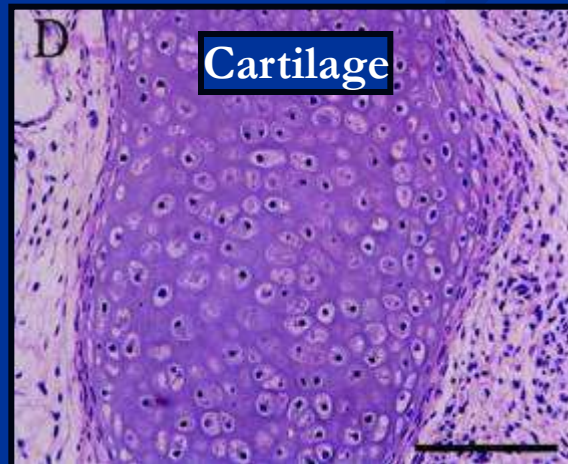
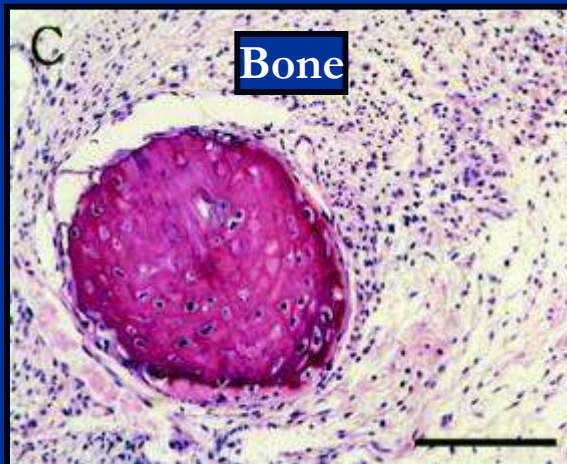
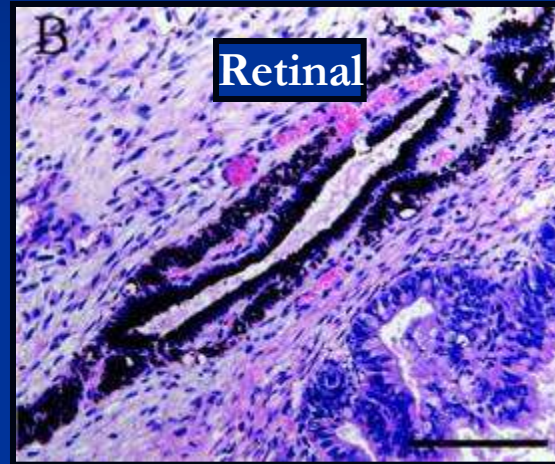
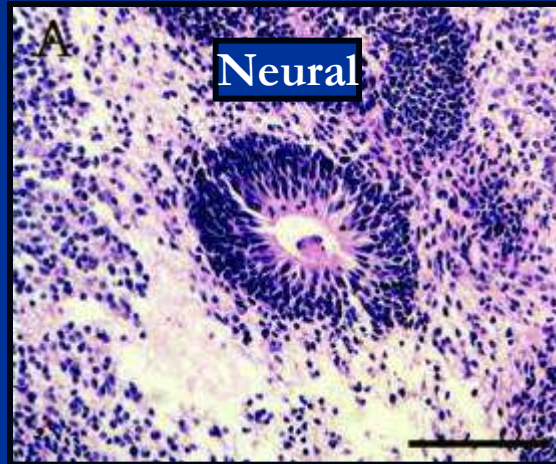
Pancreatic  
Islet

Neuron

Muscle  
cell

*Different chemicals / molecules are added to the stem cells to make them become specific types of cells.*

# Cloned ESC Differentiate Into Different Tissue Types



**IVF**

**vs.**

**SCNT**

# Stem Cells From In Vitro Fertilization (IVF)

Unused, frozen embryo,  
slated to be thrown away

Pluripotent  
stem cells



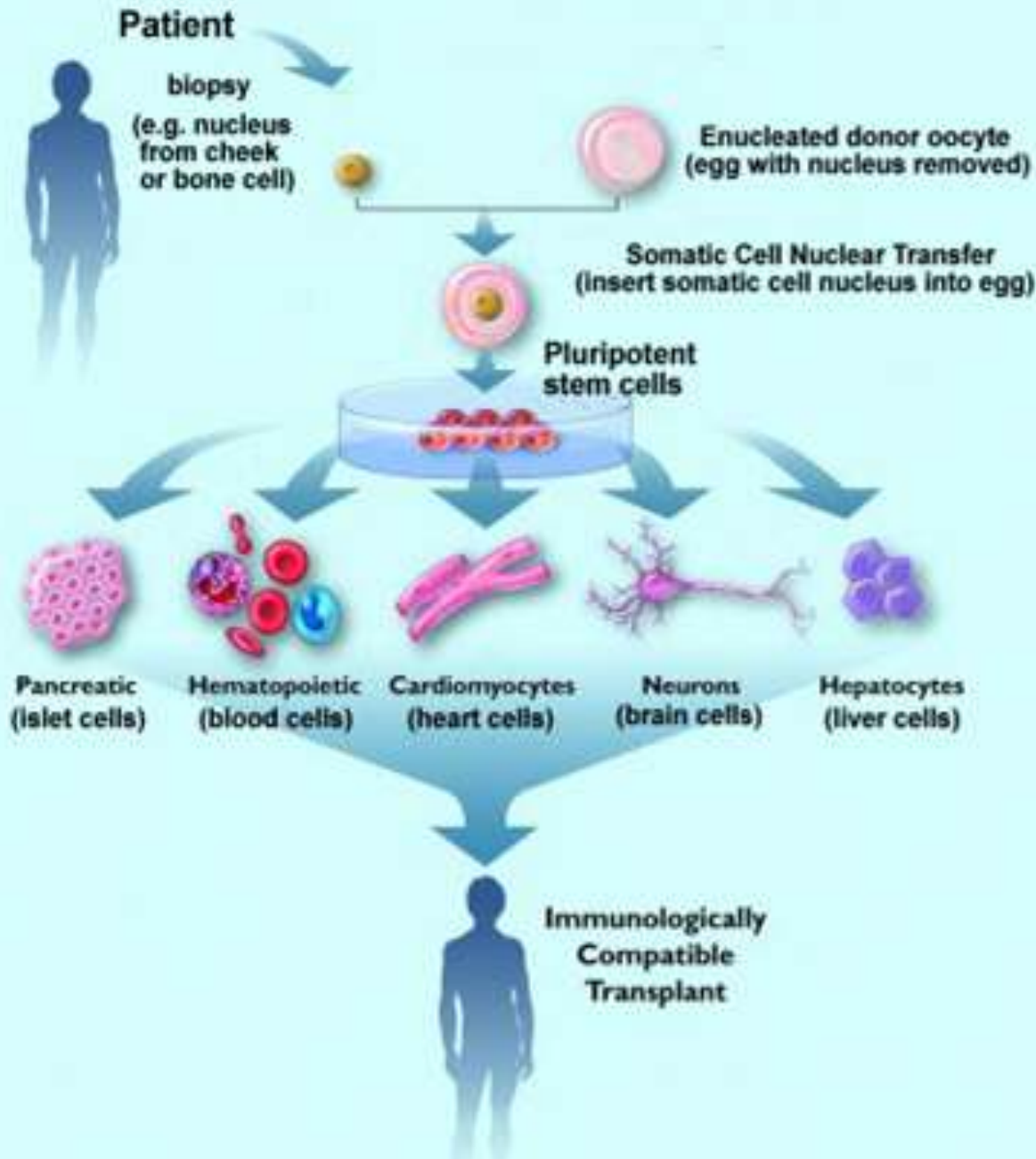
Restoration of damaged  
or destroyed tissue

Tens of thousands of frozen embryos are routinely destroyed when couples finish their treatment.

These surplus embryos can be used to produce stem cells.

Regenerative medical research aims to develop these cells into new, healthy tissue to heal severe illnesses.

# Human Therapeutic Cloning (SCNT)



## Somatic Cell Nuclear Transfer

The nucleus of a donated egg is removed and replaced with the nucleus of a mature, "somatic cell" (a skin cell, for example).

No sperm is involved in this process, and no embryo is created to be implanted in a woman's womb.

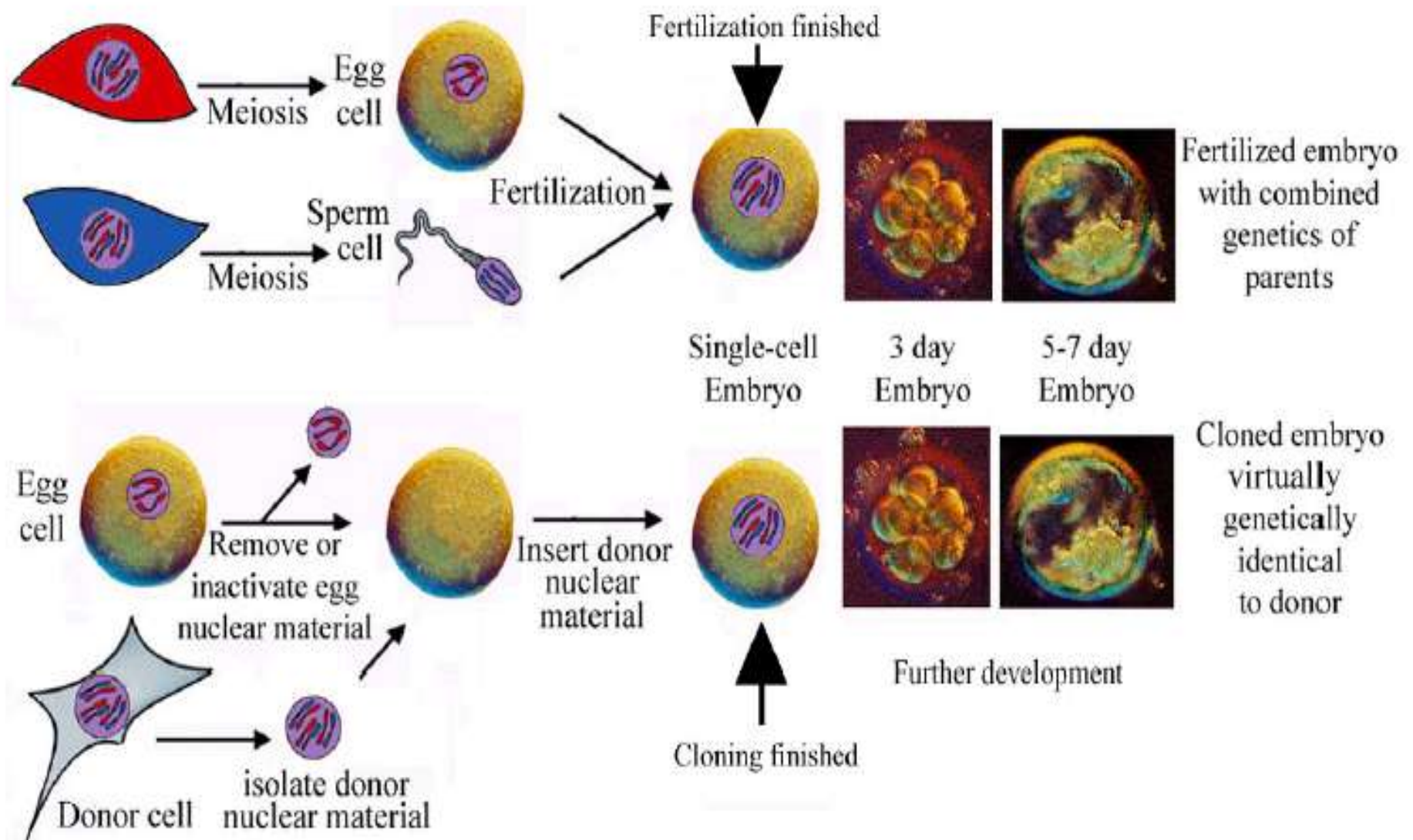
The resulting stem cells can potentially develop into specialized cells that are useful for treating severe illnesses

# Removing Nucleus

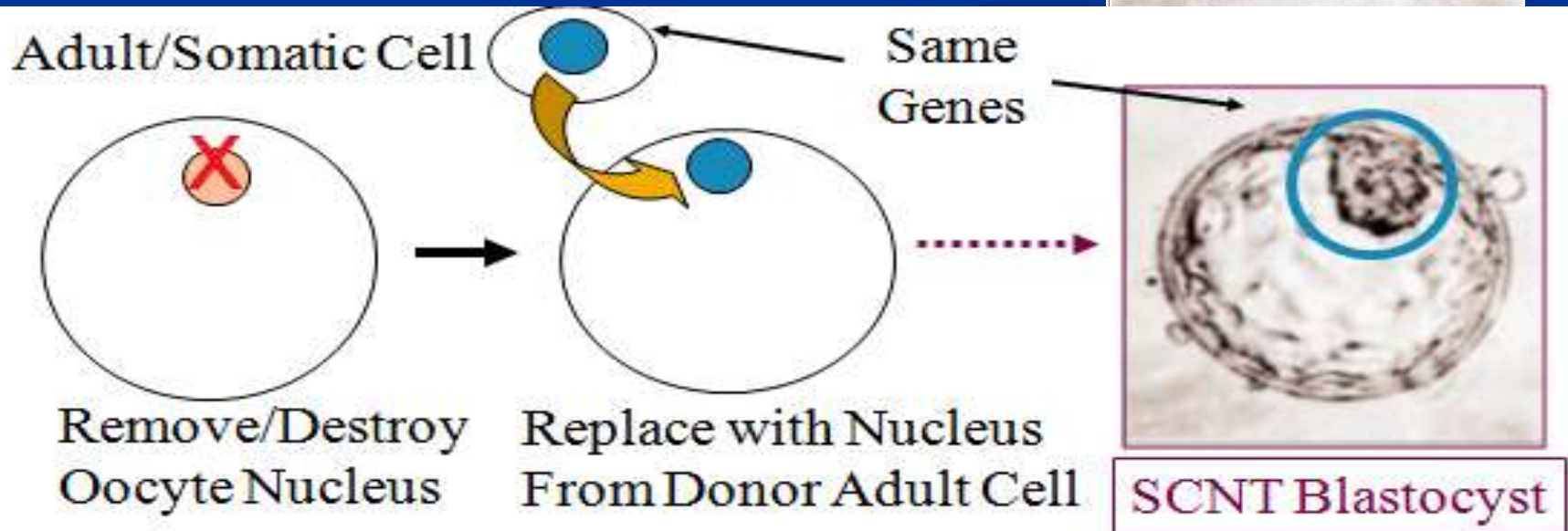
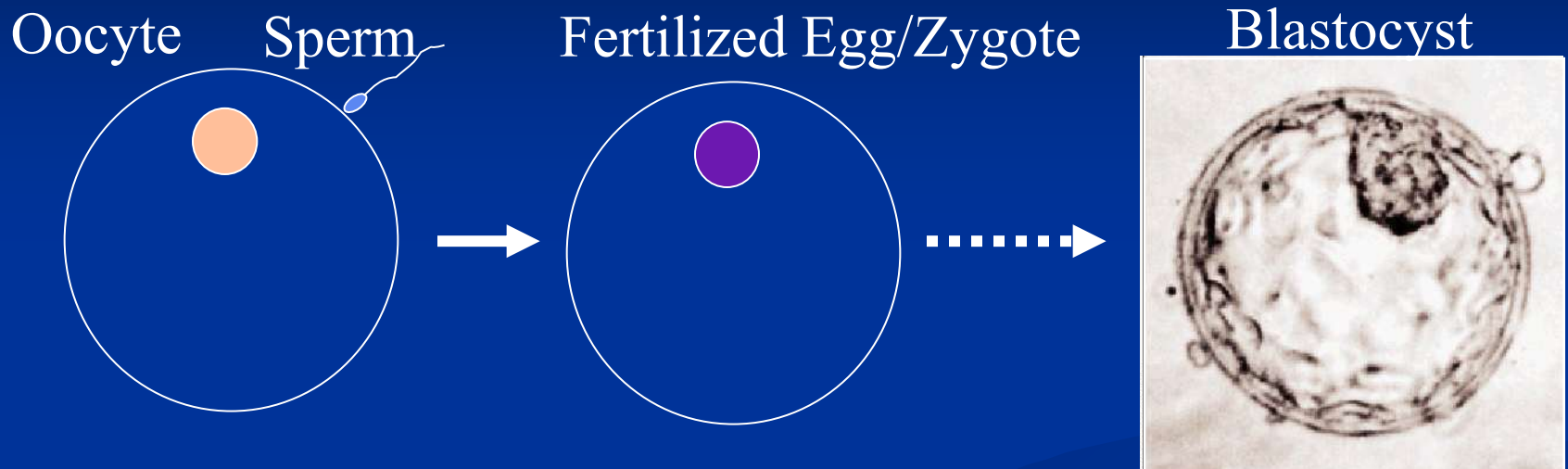


Removing the maternal nucleus before nuclear transfer

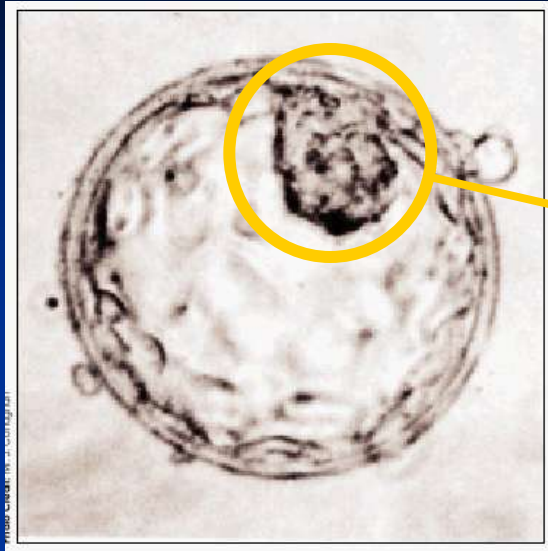
# Fertilization vs. Cloning (somatic cell nuclear transfer, SCNT)



# Somatic Cell Nuclear Transplantation (SCNT)

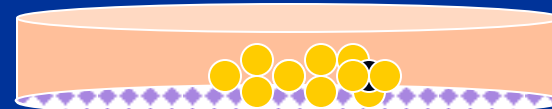


## SCNT Blastocyst



## Animal ES Cells

Inner Cell Mass



Culture in vitro



Embryonic Stem Cells  
Genetically Identical with Donor Cell

Reproductive  
Cloning



# History of Somatic Cell Nuclear Transfer (Cloning)



- 1952 – Briggs and King cloned tadpoles
- 1996 – The first mammal cloned from adult cells was Dolly, the sheep.



- 1998 – Mice cloned
- 1998 – Cows cloned
- 2000 – Pigs cloned

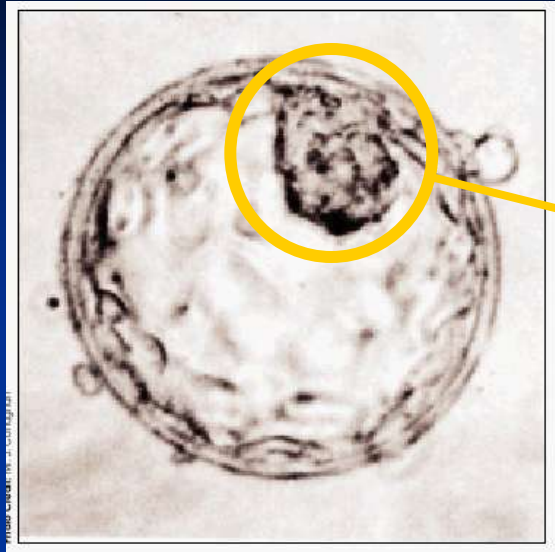


# History of Cloning

- 2001 – Cat cloned
- 2002 – Rabbits cloned
- 2003 – Mule cloned
- 2004 – Bull serial-cloned
- 2005 – Dog cloned

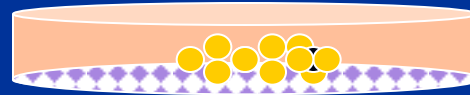


# SCNT Blastocyst

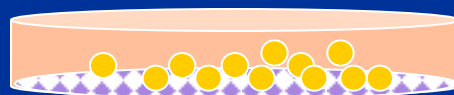


## Human ES Cells

Inner Cell Mass



Culture  
in vitro



Differentiated  
Cell Types

Embryonic Stem Cells  
Genetically Identical with Donor Cell



# How Successful Was Animal Cloning? Very low (~1-3%)

Dolly ( <b>sheep</b> )	1 live birth out of 29 cloned embryos	3%
Cloned <b>mice</b>	31 live births out of 2468 cloned embryos	1%
Cloned <b>pigs</b>	5 live births out of 335 cloned embryos	1%
Cloned <b>goats</b>	3 live births out of 85 cloned embryos	3%
Cloned <b>cattle</b>	30 live births out of 496 cloned embryos	6%
Cloned <b>cat</b>	1 live birth out of 87 cloned embryos	1%
Cloned <b>rabbits</b>	6 live births out of 371 of cloned embryos	1%

# Fetal stem cells

- Fetal stem cells are primitive cell types found in the organs of fetuses

# New Frontiers: Alternative Sources of Pluripotent Stem Cells from Amniotic Fluid

washingtonpost.com

## Scientists See Potential In Amniotic Stem Cells

They Are Highly Versatile And Readily Available

By Rick Weiss

Washington Post Staff Writer

Monday, January 8, 2007; A01

A type of cell that floats freely in the amniotic fluid of pregnant women has been found to have many of the same traits as embryonic stem cells, including an ability to grow into brain, muscle and other tissues that could be used to treat a variety of diseases, scientists reported yeste



# Umbilical cord stem cells

- Umbilical cord blood
- Umbilical cord matrix (Wharton jelly)



# Placenta a Source of Stem Cells

- Placental stem cells, like umbilical cord blood and bone marrow stem cells, can be used to cure chronic blood-related disorders such as sickle cell disease, Thalassaemia, and leukaemia.



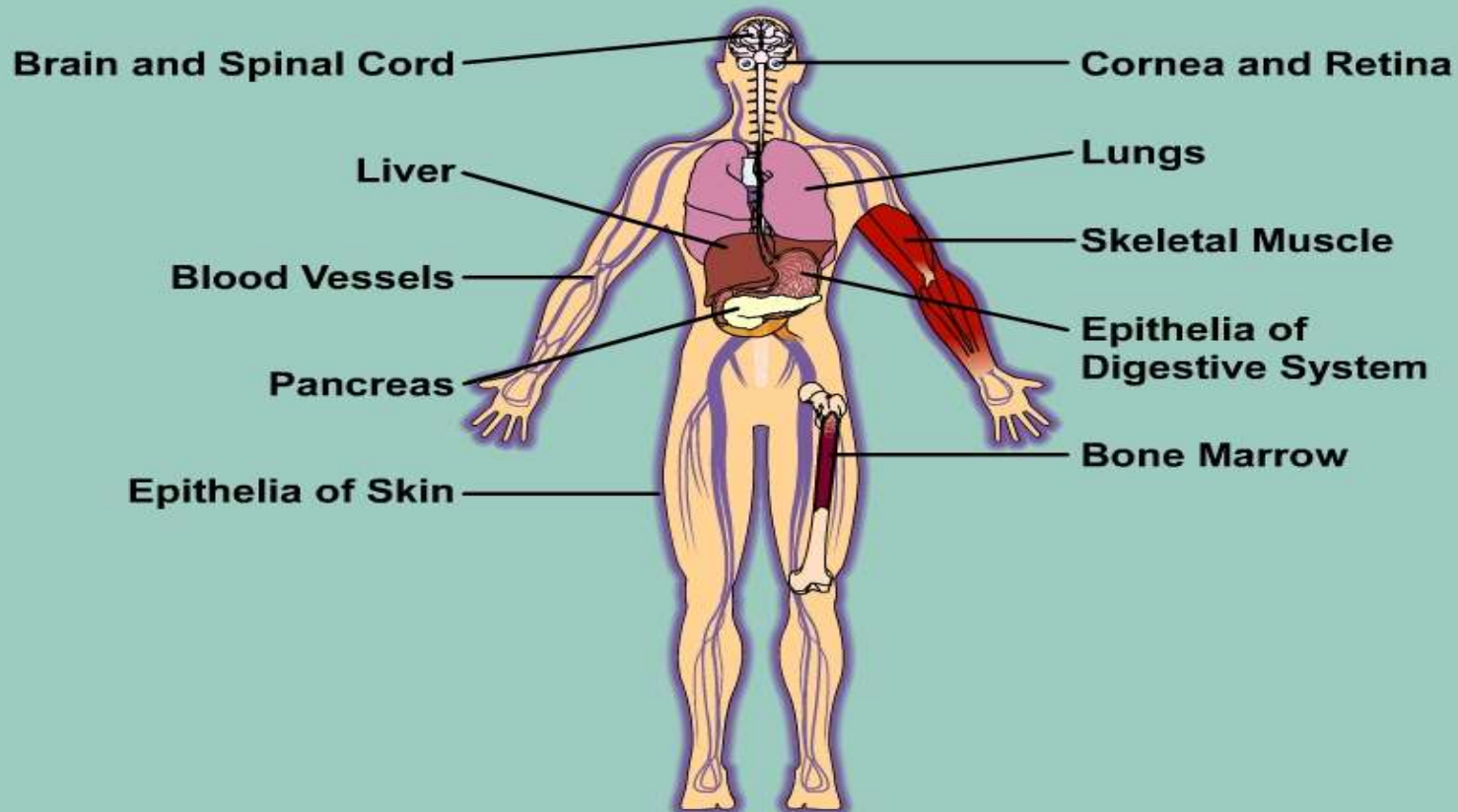
## Adult stem cells

- Define as multipotent
- Adult stem cells have been isolated from nearly every tissue

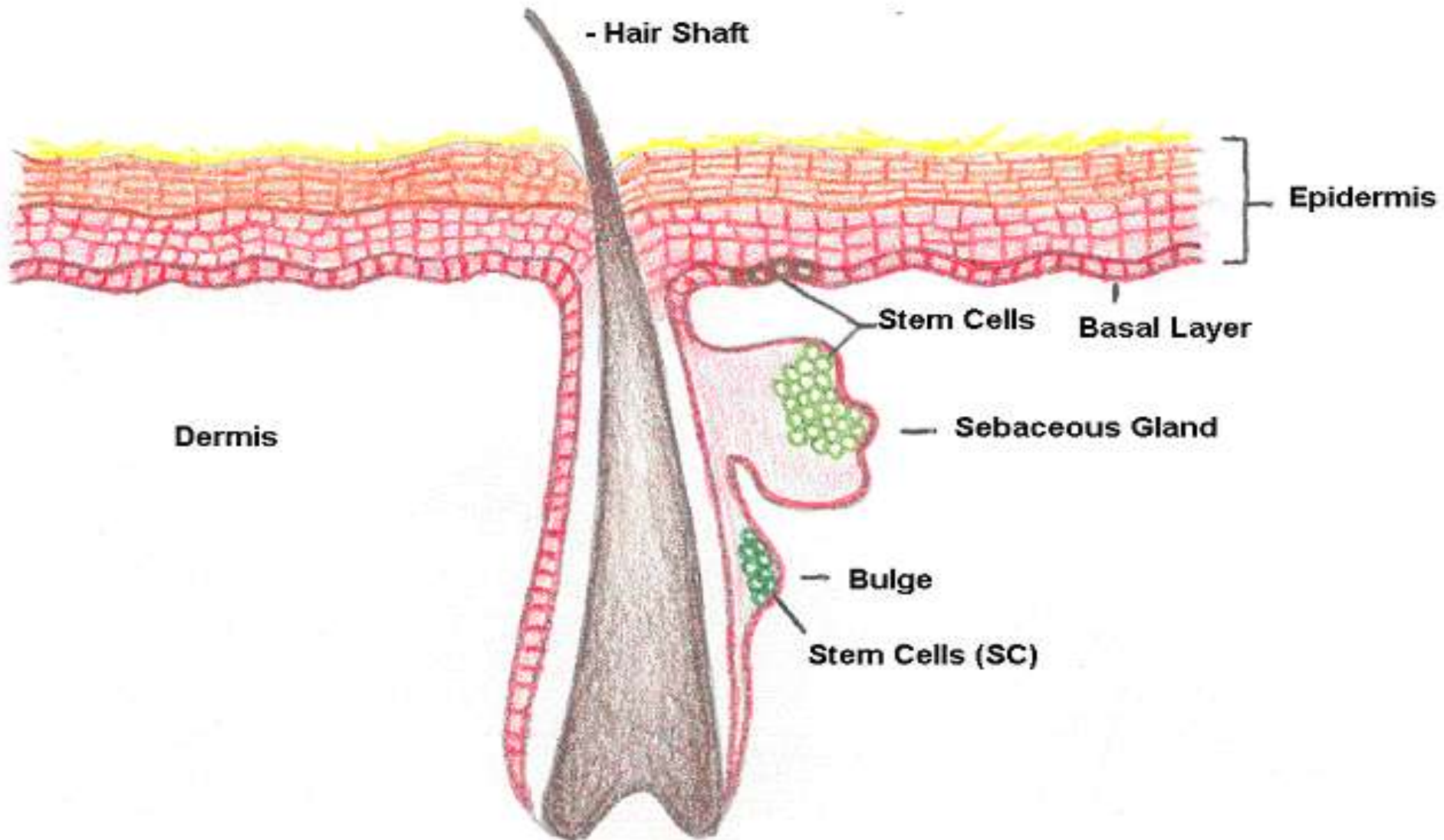
Eg. bone marrow, adipose tissue, brain,  
Dental pulp, intestine, skin.....

# STEM CELLS HAVE ALSO BEEN FOUND IN “MATURE” ORGANS

## Adult Stem Cell Locations



# Adult Stem Cells



# Main types of Adult stem cells

- Hematopoietic stem cells
- Mesenchymal stem cells
- Neural stem cells

# Hematopoietic stem cells

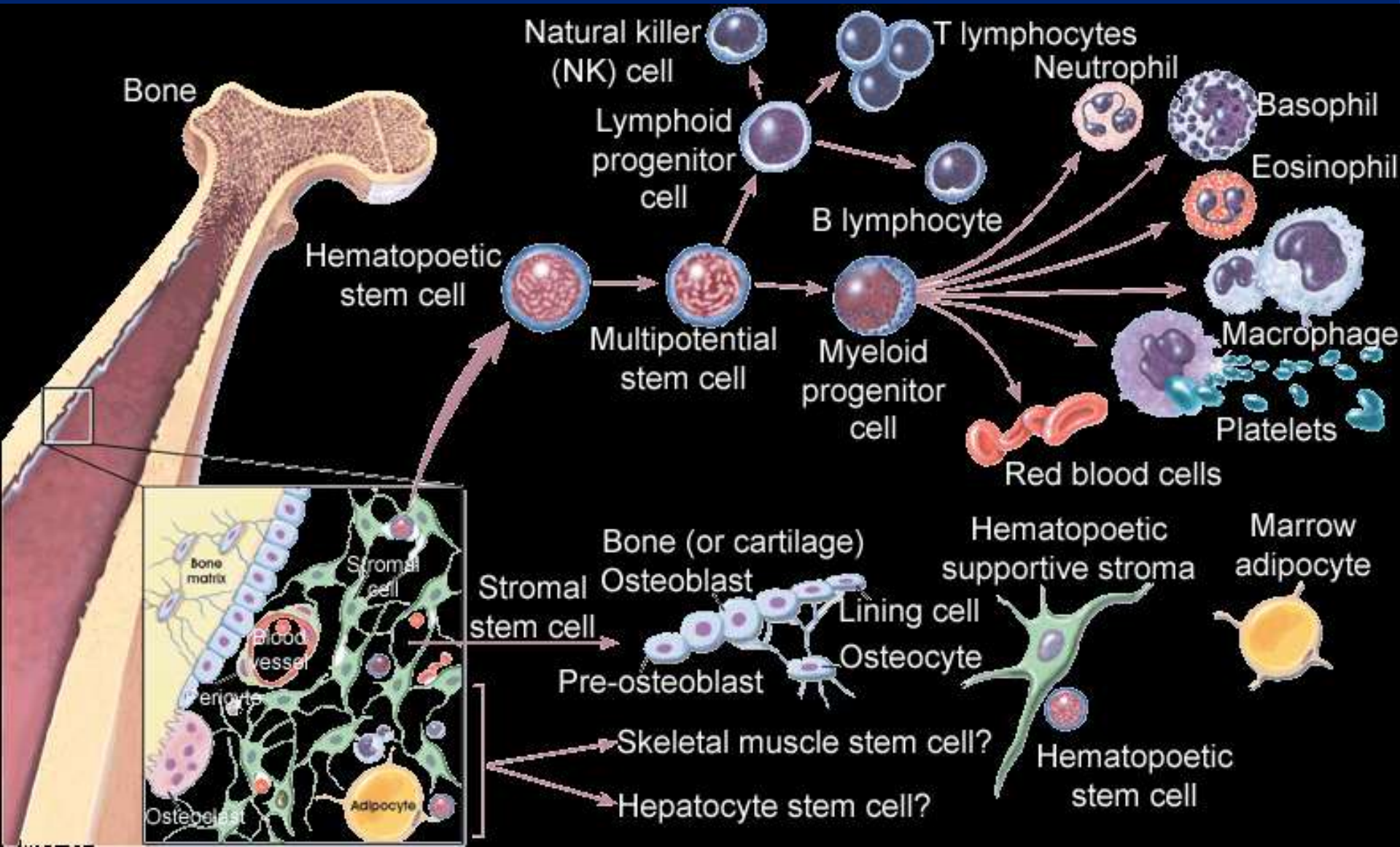
- Source:

mainly bone marrow , peripheral blood & cord blood.

- Differentiate :

to other blood cells

# Bone Marrow Stem Cells



# Mesenchymal stem cells

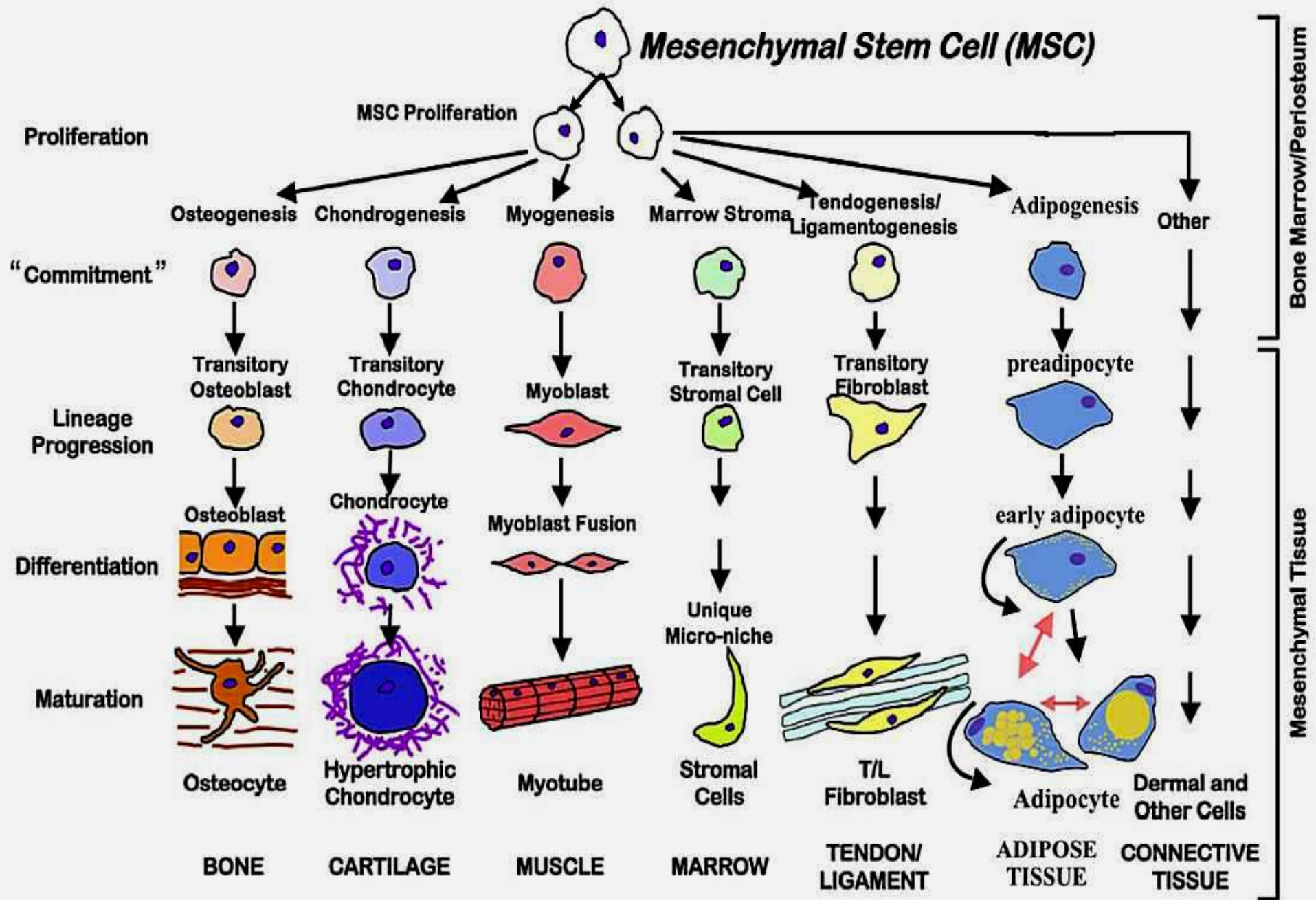
## ■ Source:

bone marrow , adipose tissue ,  
Dental pulp.....

## ■ Differentiate :

to osteocyte , adipocyte ,  
chondrocyte,..

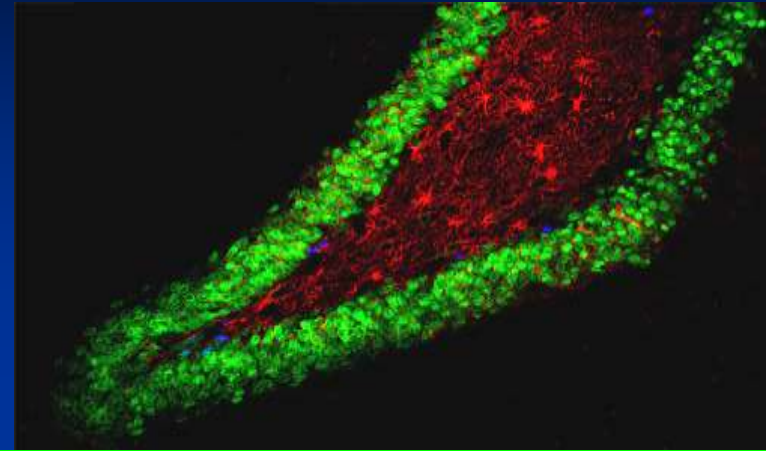
# THE MESENGENIC PROCESS



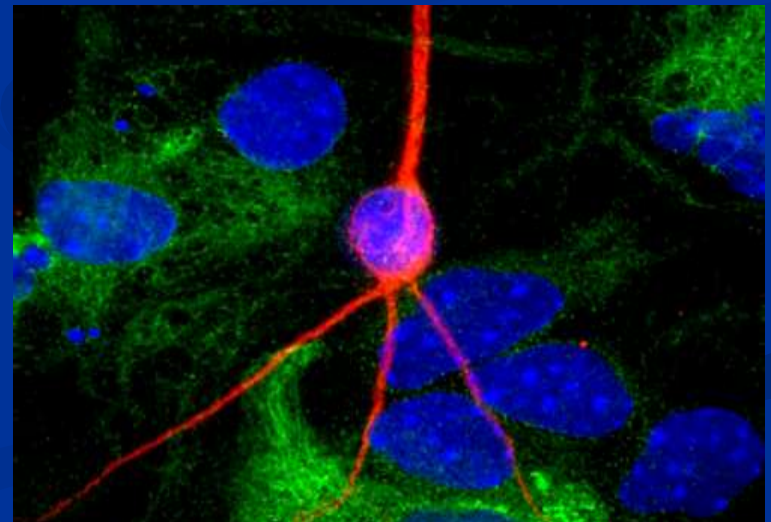
# Neural stem cells

## ■ Source:

- 1- Subventricular zone lining the lateral ventricle, where they give rise to newly-born neurons that migrate to the olfactory bulb via the rostral migratory stream.
- 2-subgranular zone, part of the dentate gyrus of the hippocampus



Section of the hippocampus, blue dots are neural stem cells



Mature neuron (red)

# PLURIPOTENCY

Stem cells retain the ability to differentiate into cells and tissues from all **3 germ layers** (endoderm, mesoderm, and ectoderm).

But, these (pluripotent) cells **cannot form** the other 'extra- embryonic' tissues such as placenta and membranes, necessary for complete development,

therefore they cannot give rise to a complete new individual.

# Induced Pluripotent stem cells (**iPS**)



**Nobel Prize  
in Medicine**

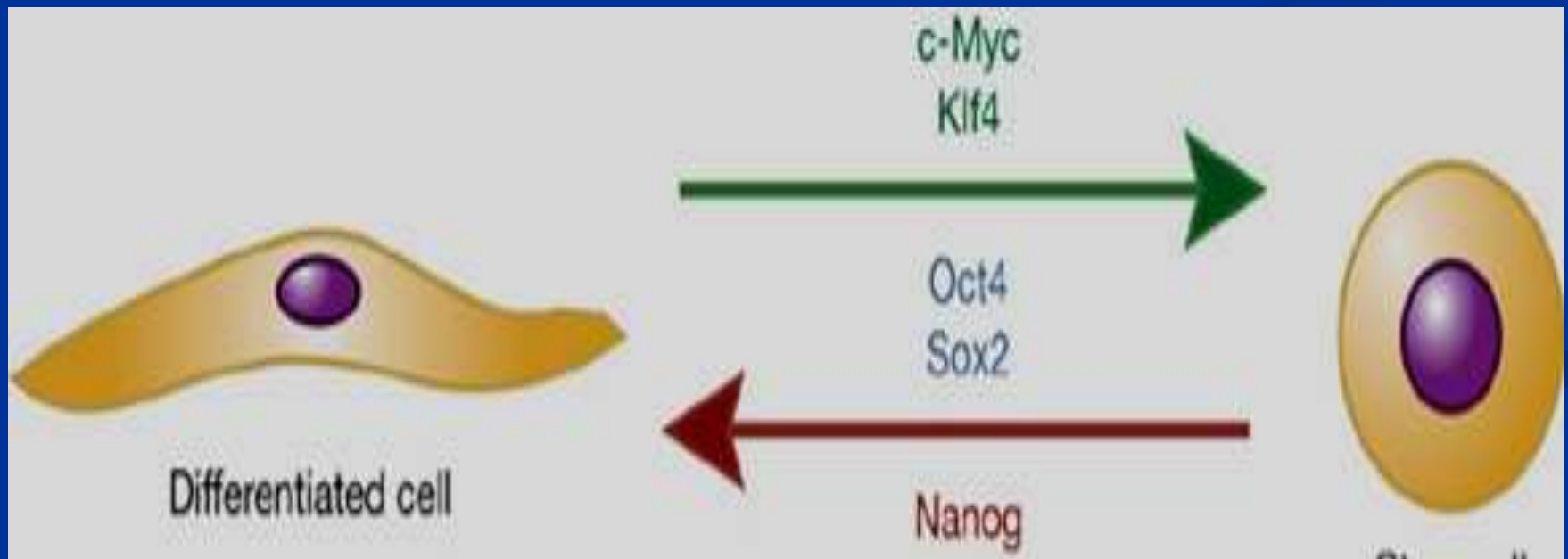
**2012**



Shinya Yamanaka (right) and John B Gurdon (left), the winners of the

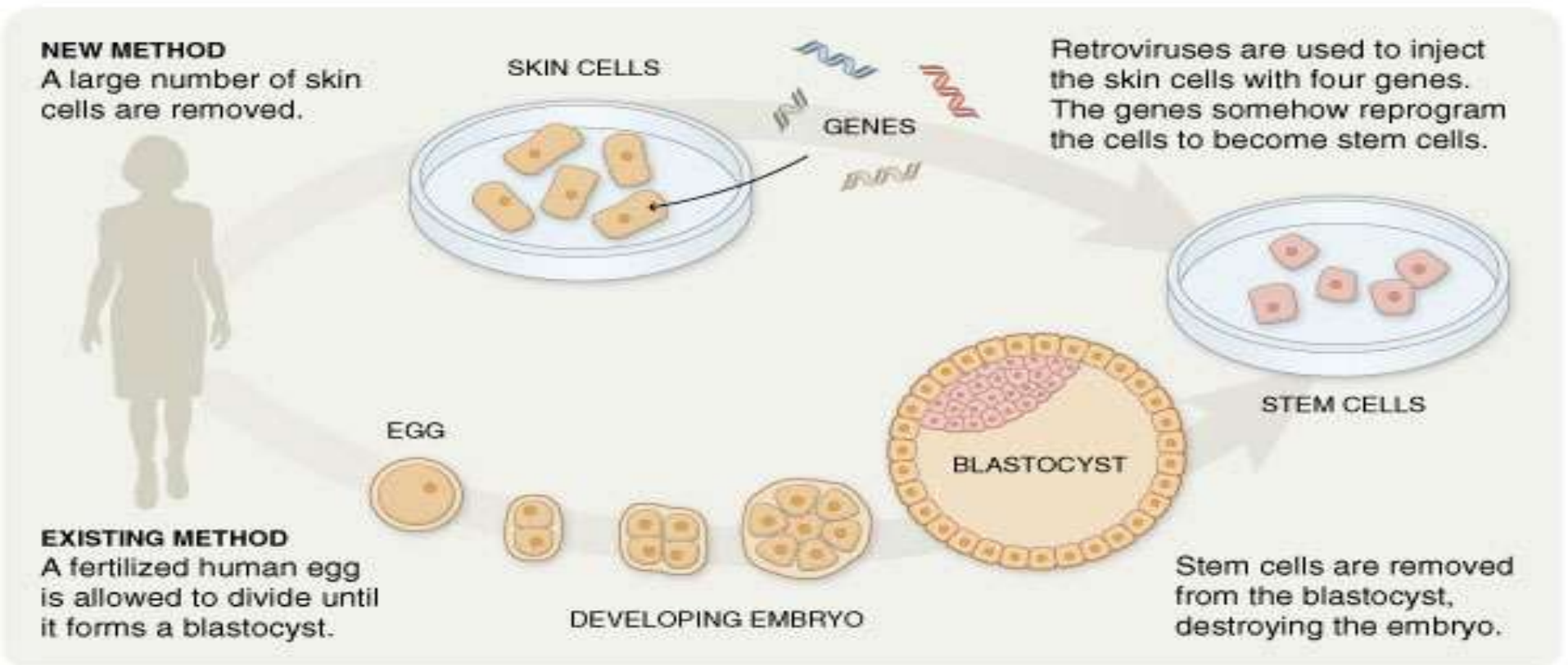
# Induced Pluripotent stem cells (**iPS**)

- Transfection of certain stem cell-associated genes into adult cell such as fibroblast .



# Reprogramming Human Skin Cells

Researchers have developed a technique for creating stem cells without the controversial use of human eggs or embryos. If the method can be perfected, it could quell the ethical debate troubling the field.



## TIMELINE

1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
<b>July 1995</b> Congress bans federal financing of research on human embryos.		<b>July 1996</b> Dolly is born. The lamb is the first clone of an adult mammal.		<b>Nov. 1998</b> First isolation and cultivation of embryonic stem cells. The cells are derived from fertilized human eggs.		<b>Aug. 2001</b> President Bush announces that federal money will pay for research on existing stem cell lines, but not new lines.			<b>Nov. 2004</b> California voters approve a measure to spend \$3 billion over 10 years on embryonic stem cell research.			<b>Nov. 2007</b> New Jersey voters reject a measure to borrow \$450 million for stem cell research.

# Pros and Cons to iPS cell technology

## ■ Pros:

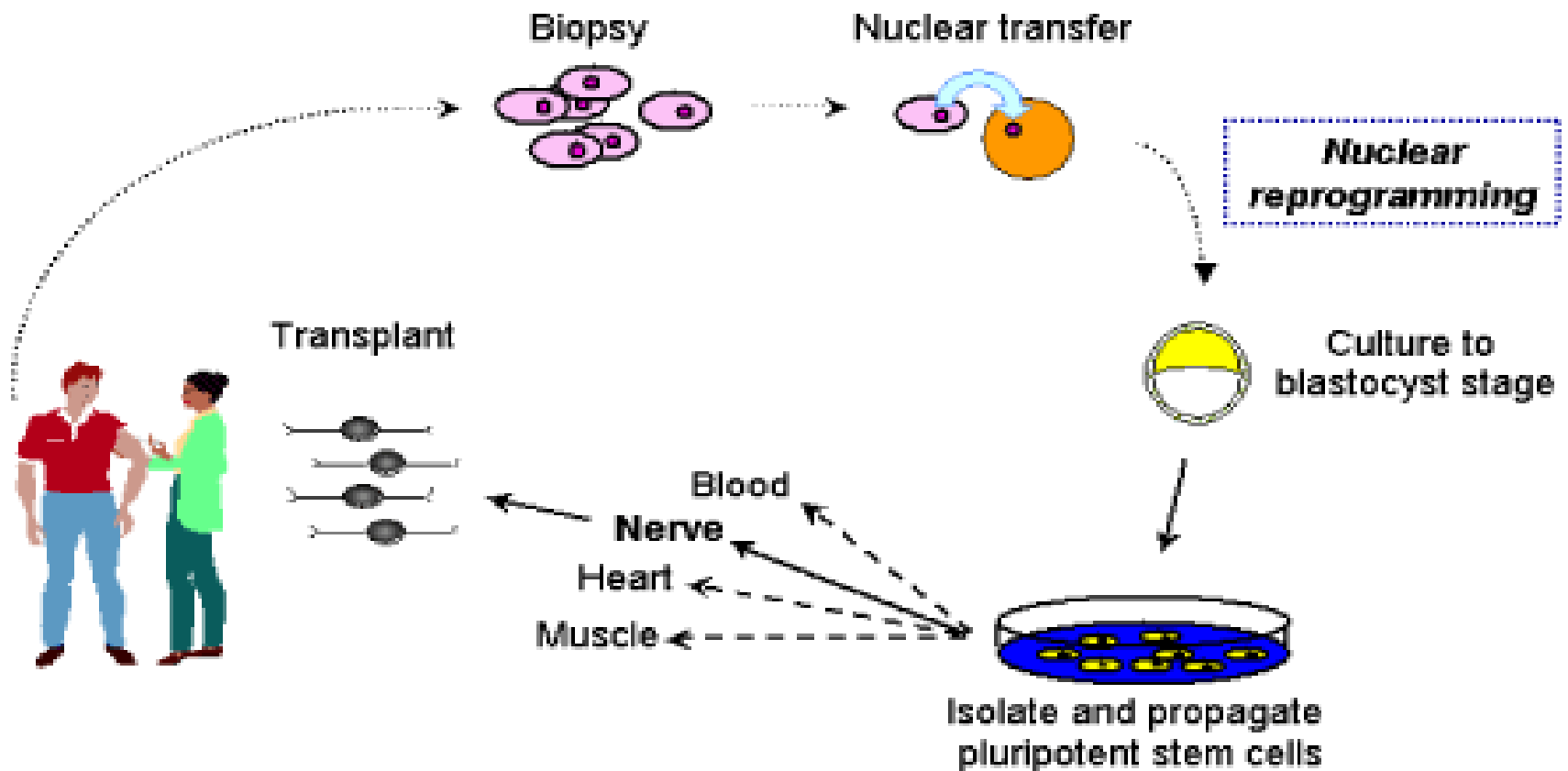
- Cells would be genetically identical to patient or donor of skin cells (no immune rejection!)
- Do not need to use an embryo

## ■ Cons:

- Cells would still have genetic defects
- One of the pluripotency genes is a cancer gene
- Viruses might insert genes in places we don't want them (causing mutations)

# *Treatments becomes Specific*

## Patient-Specific Stem Cell Therapy



# What is Tissue Engineering ( TE )?

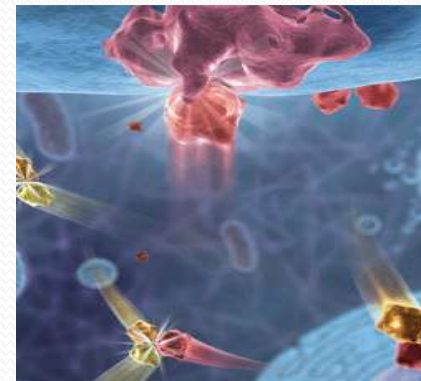
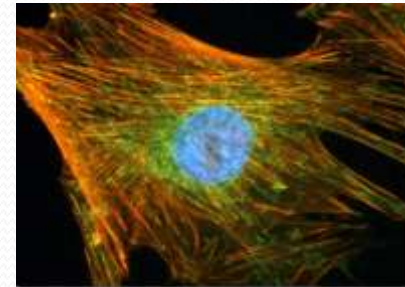
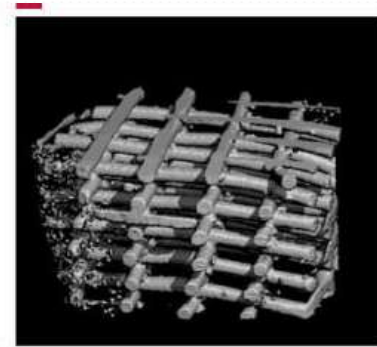
- TE is an interdisciplinary field that applies the principles of engineering and the life sciences towards the development of biological substitutes that restore, maintain, or improve tissue function.

OR

- Developing living tissue using cells, biomaterials, and signaling molecules

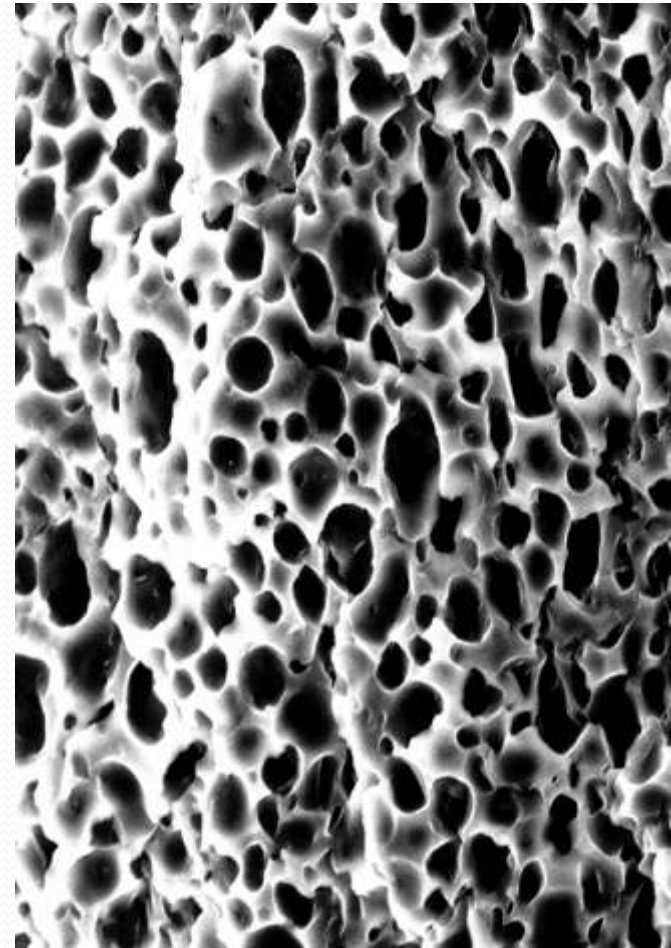
# Tissue Engineering (TE)

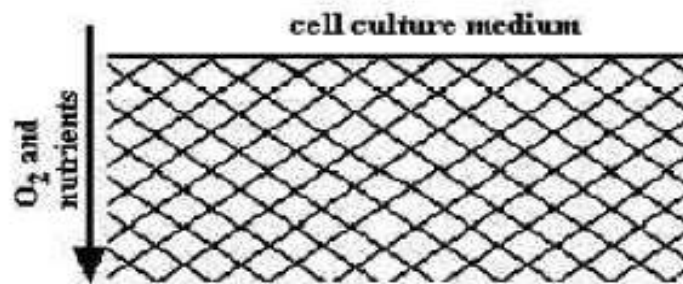
- Scaffolds  
Biomaterials, which may be natural or artificially derived, providing a platform for cell function, adhesion and transplantation
- Cells  
Any class of cell, such as stem or mesenchymal cell
- Signals  
Proteins and growth factors driving the cellular functions of interest
- Bioreactor  
System that supports a biologically active environment (ex. Cell culture)



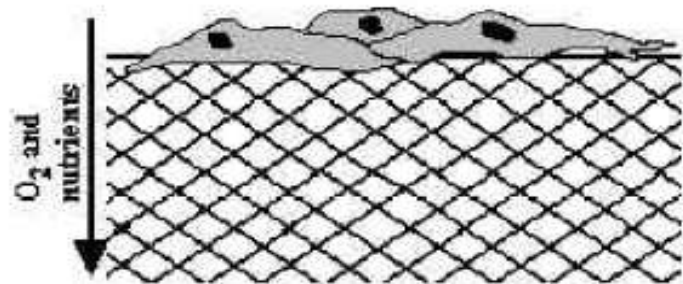
# The Scaffold

- Cells are often implanted into an artificial structure that is able to maintain the structure for tissue formation. This artificial structure is a scaffold
- Scaffolds must: Allow cell attachment and migration, enable transportation of vital cell nutrients, and be biodegradable
- Stem cells are seeded into a scaffold. The scaffold is then implanted into the correct position. A stimulus carried by the scaffold triggers the cells to divide. The scaffold provides nutrients and structure while the cells divide.
- The scaffold biodegrades as the cells start to form a structure strong enough to support itself.

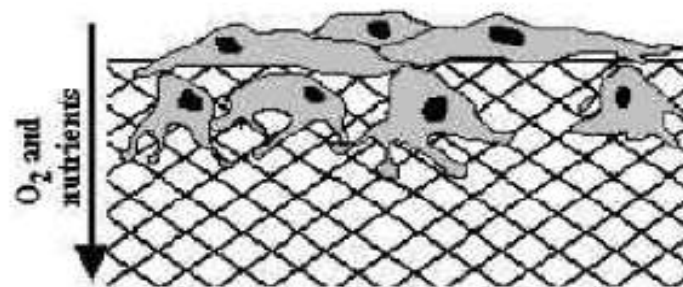




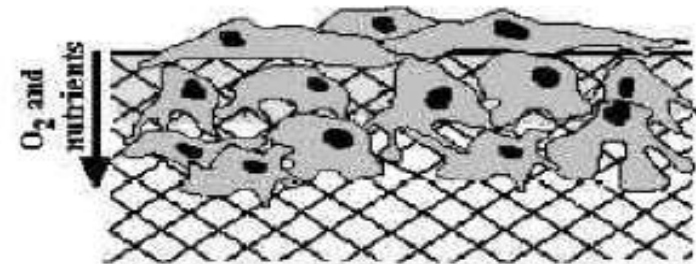
a) Tissue engineering scaffold which is an open-cell foam structure. Oxygen and nutrients are supplied from the liquid cell culture medium.



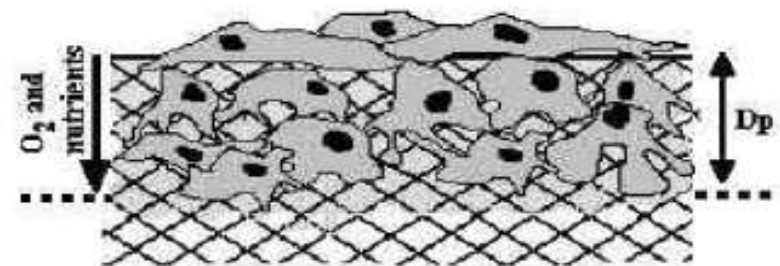
b) Cell seeding on scaffold.



c) Cells start to proliferate and migrate into the pores of the scaffold.



d) The cells fully colonise the pores and start to lay down their own extracellular matrix.



e) The top layer of cells consumes most the oxygen and nutrients in addition to limiting the diffusion of these components, thus reducing the amount available for pioneering cells migrating deep into the scaffold. Eventually, cellular migration is halted due to the lack of oxygen and nutrients supply. The layer of cells that can survive on the diffusion of oxygen and nutrients from the medium is called the cellular penetration depth ( $D_p$ ).

(Figure 4) Diagram of how a conventional scaffold operates, and how it is insufficient to support large scale growth.

# Natural Scaffolds

- **Protein-based biomaterials**

- Collagen
- Fibrin
- Silk

- **Polysaccharide-based biomaterials**

- Agarose
- Alginate
- Hyaluronan
- Chitosan



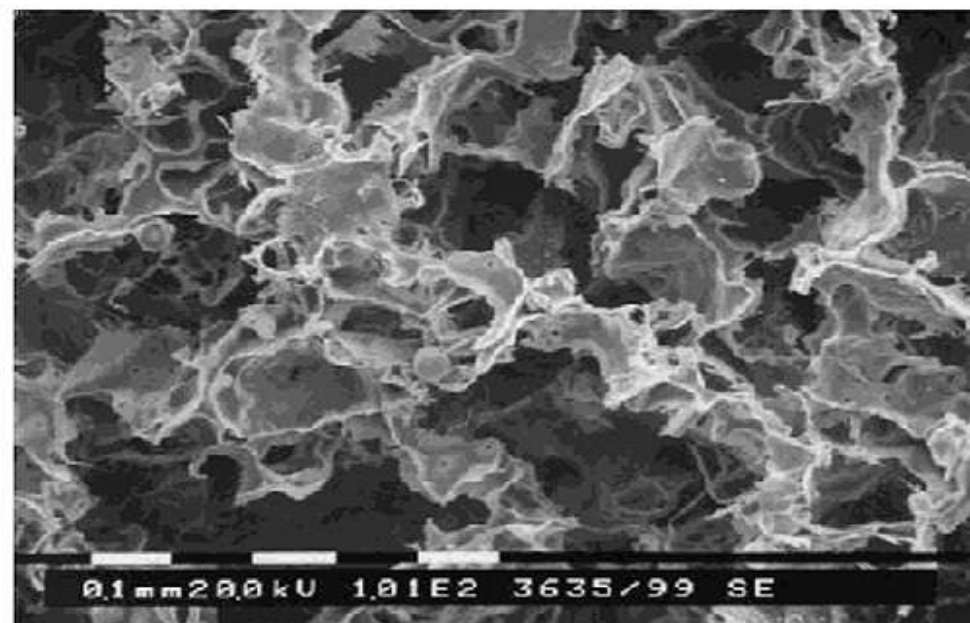
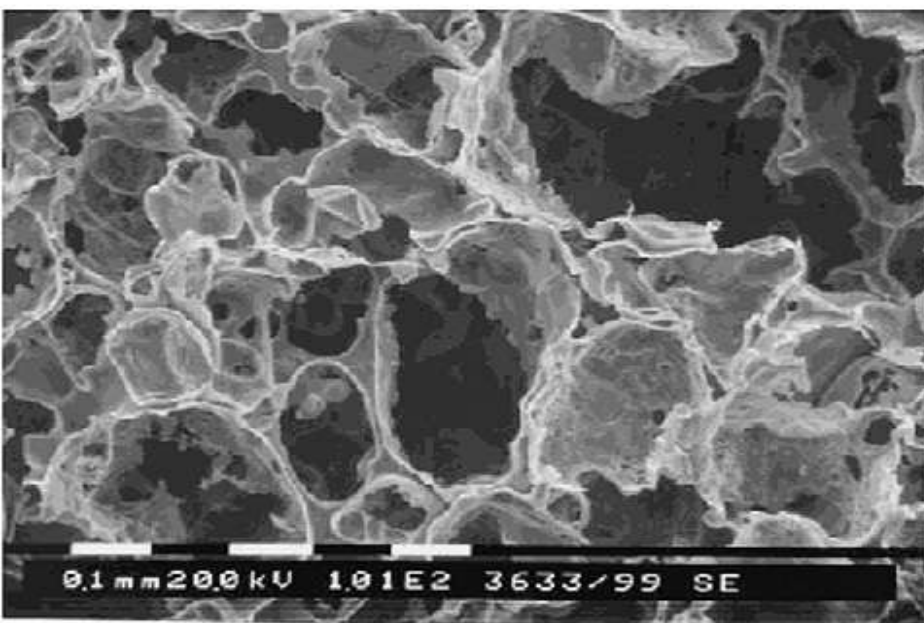
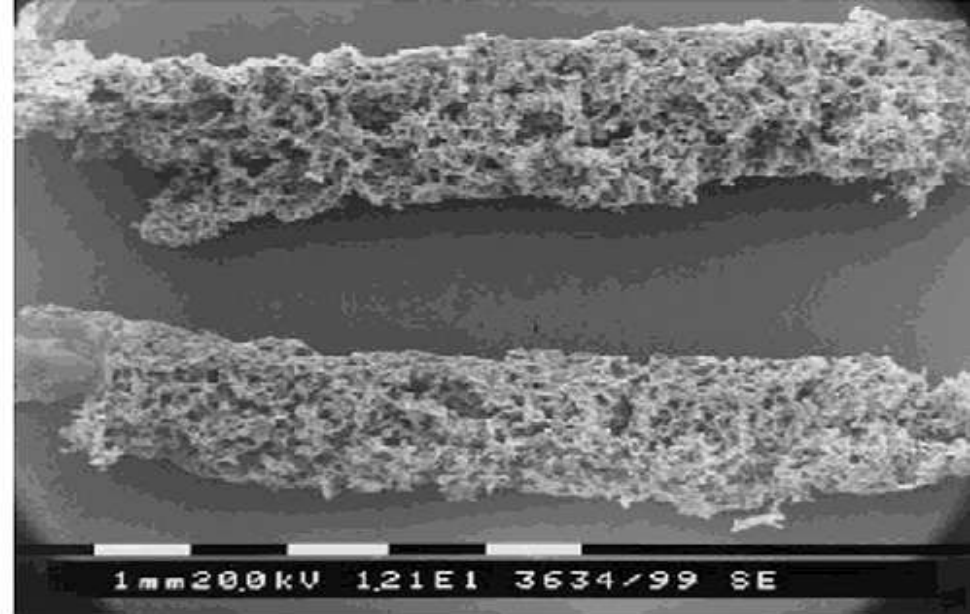
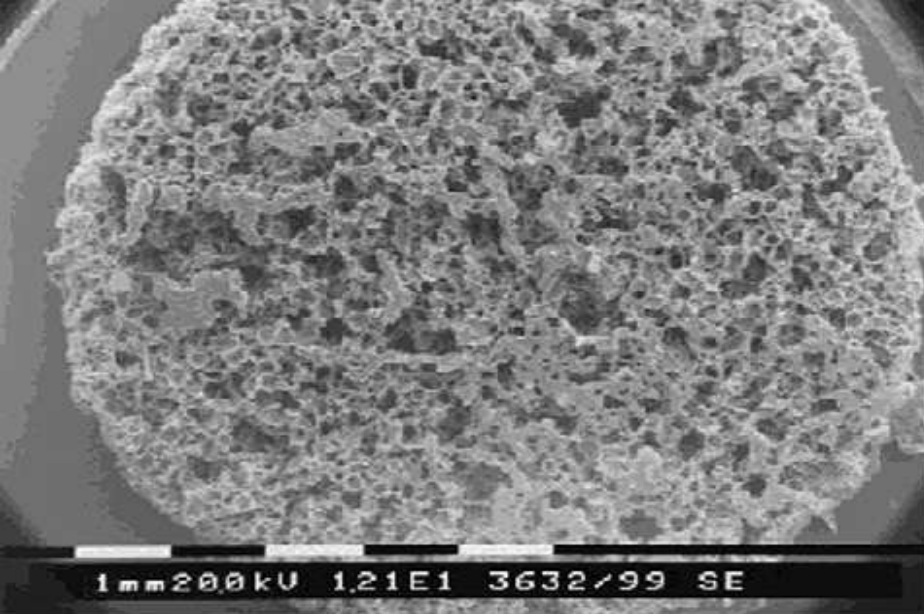
# Synthetic Scaffolds

- **Polymer-based biomaterials**
  - Poly (lactic-co-glycolic acid)
  - Poly (ethylene glycol)
- **Peptide-based biomaterials**
- **Ceramic-based biomaterials**

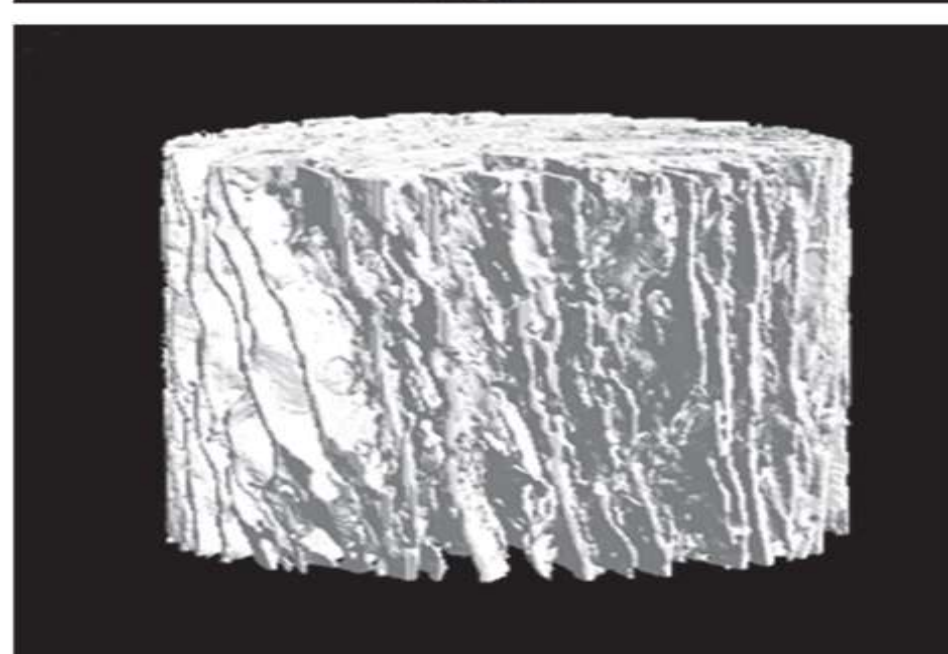
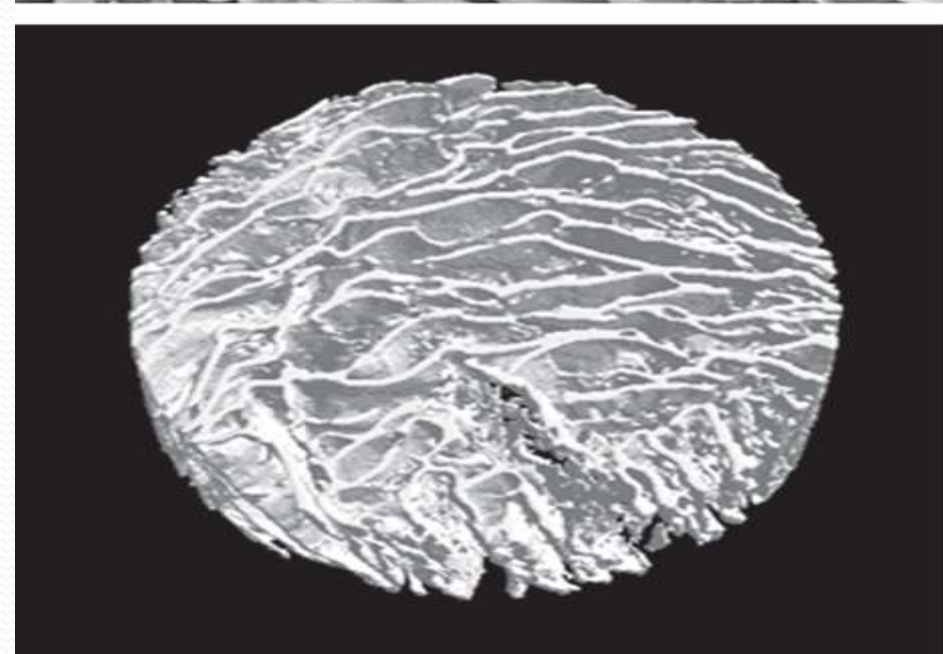
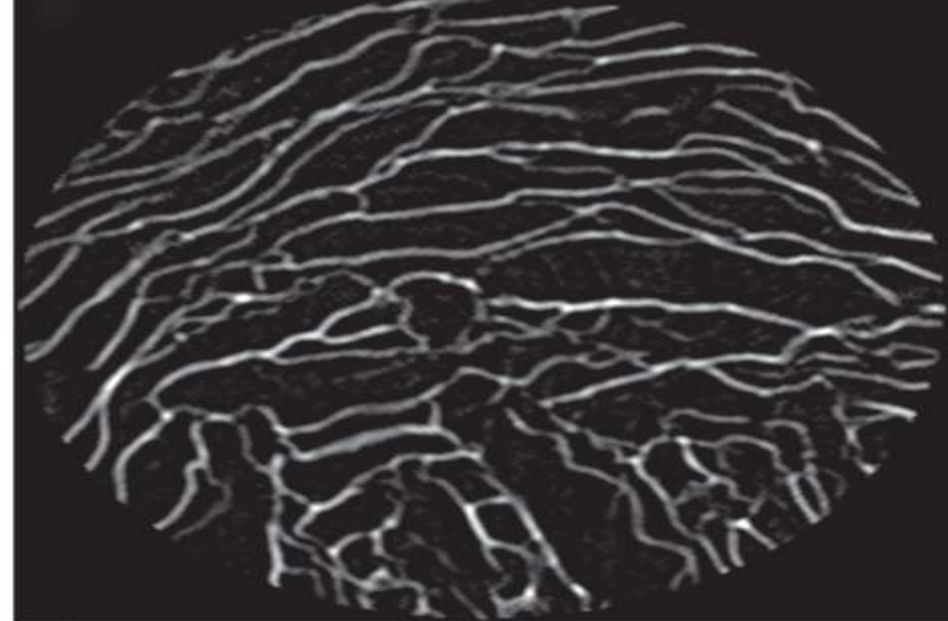
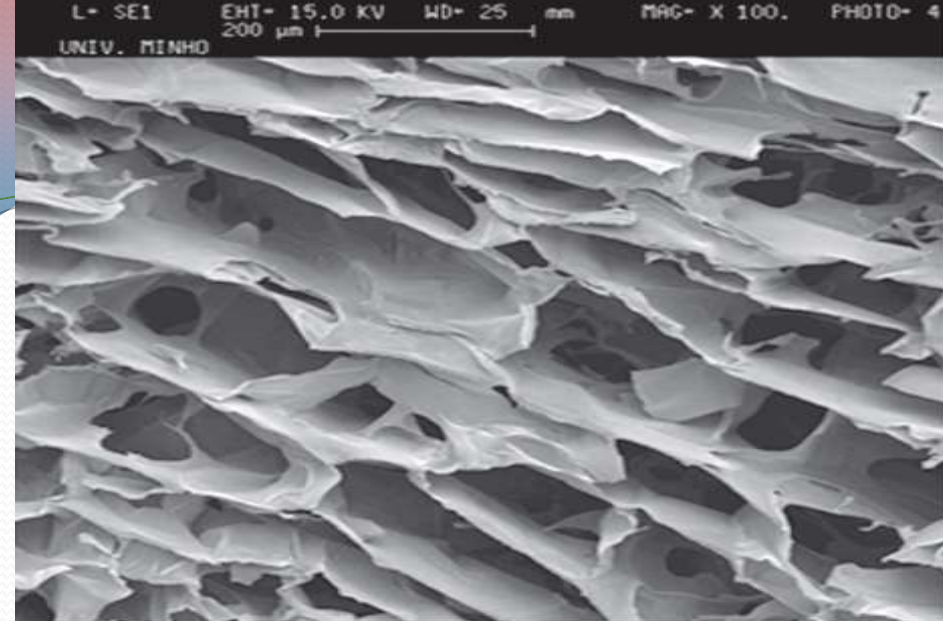


Ideal scaffolds have been identified as having the following properties:

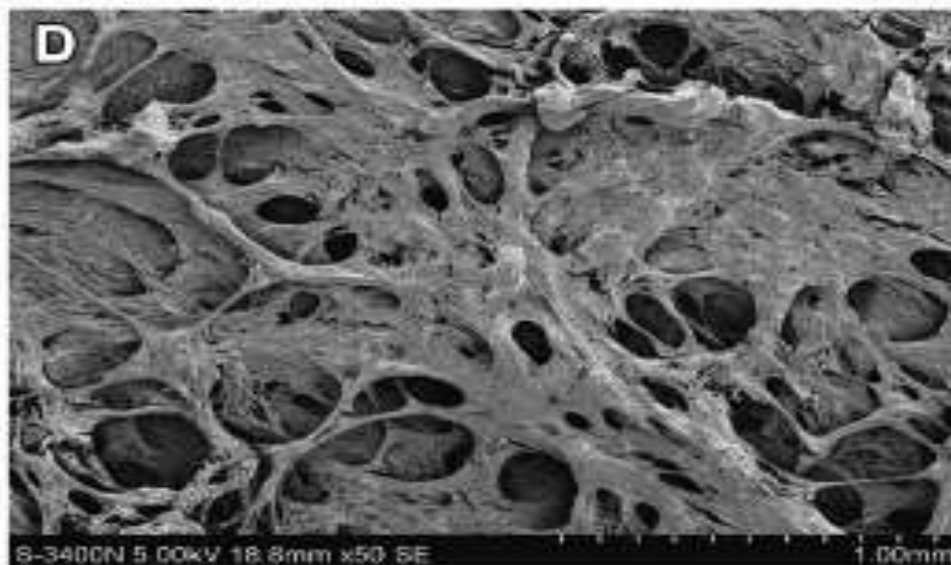
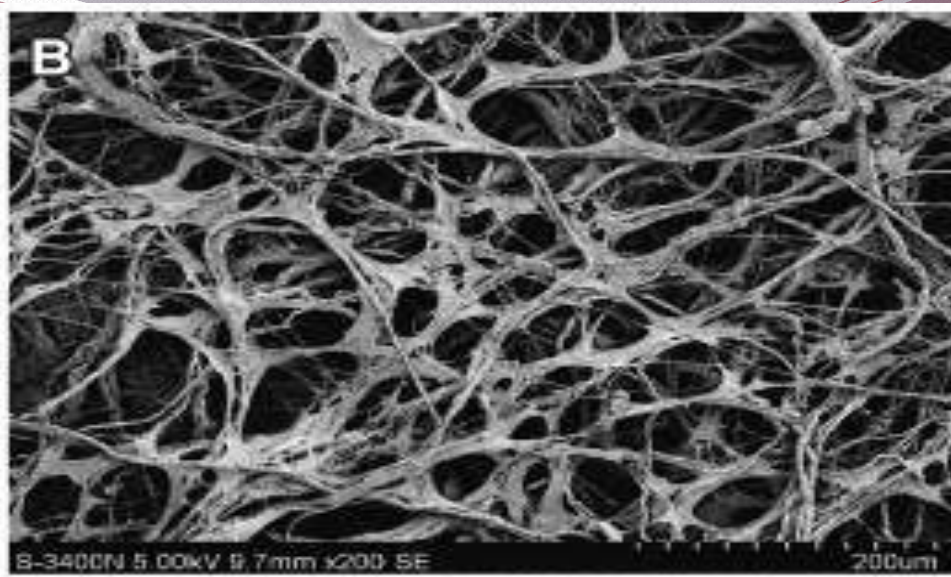
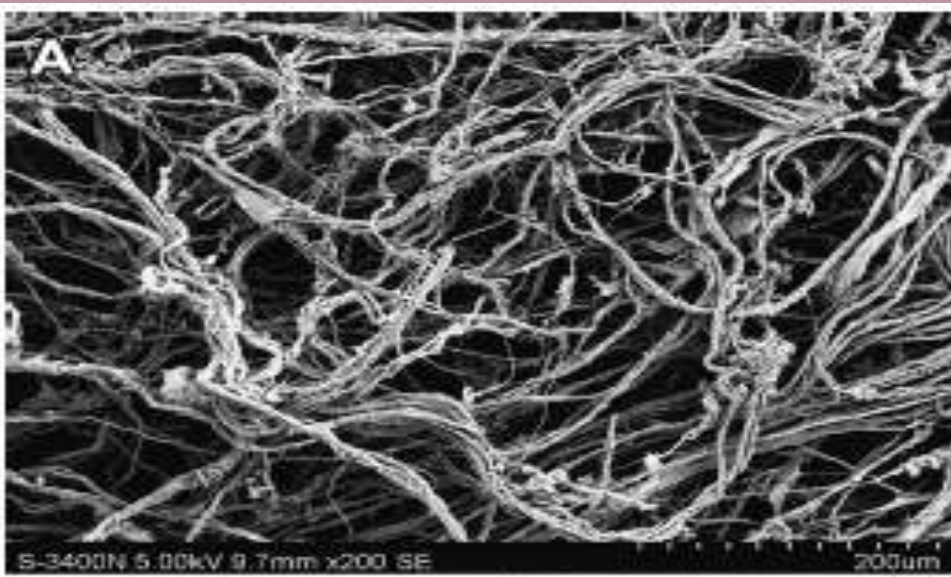
- (1) porous and permeable to allow cell growth, migration, and interaction, as well as the transport of nutrients,
- (2) biodegradable to match *in vivo/in vitro tissue growth with nontoxic*, easily eliminated by products,
- (3) biocompatible and with a surface chemistry suitable for promoting cell attachment, proliferation, and differentiation,
- (4) mechanically favorable to support cellular organization, and
- (5) shapeable, to conform to different geometries and sizes.



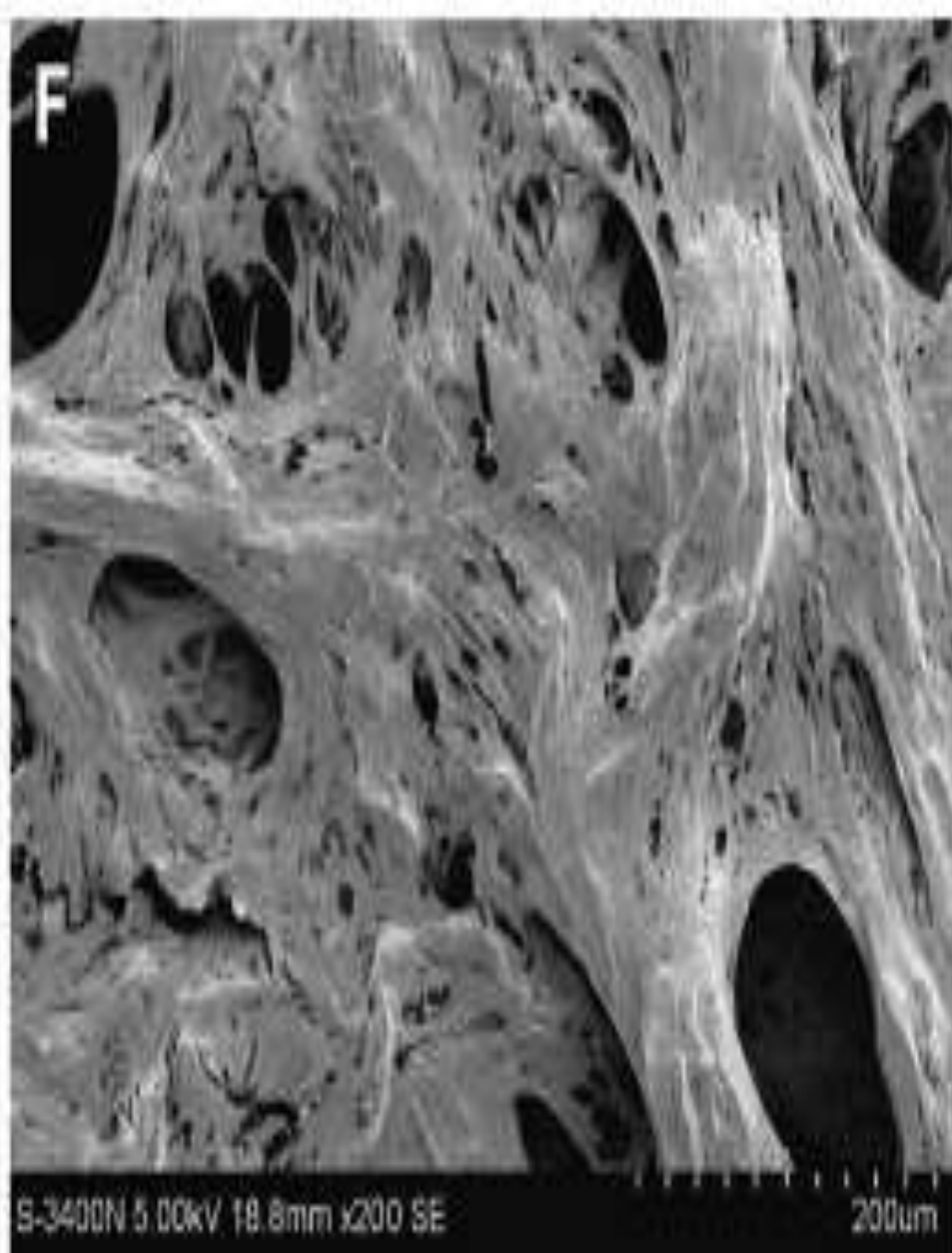
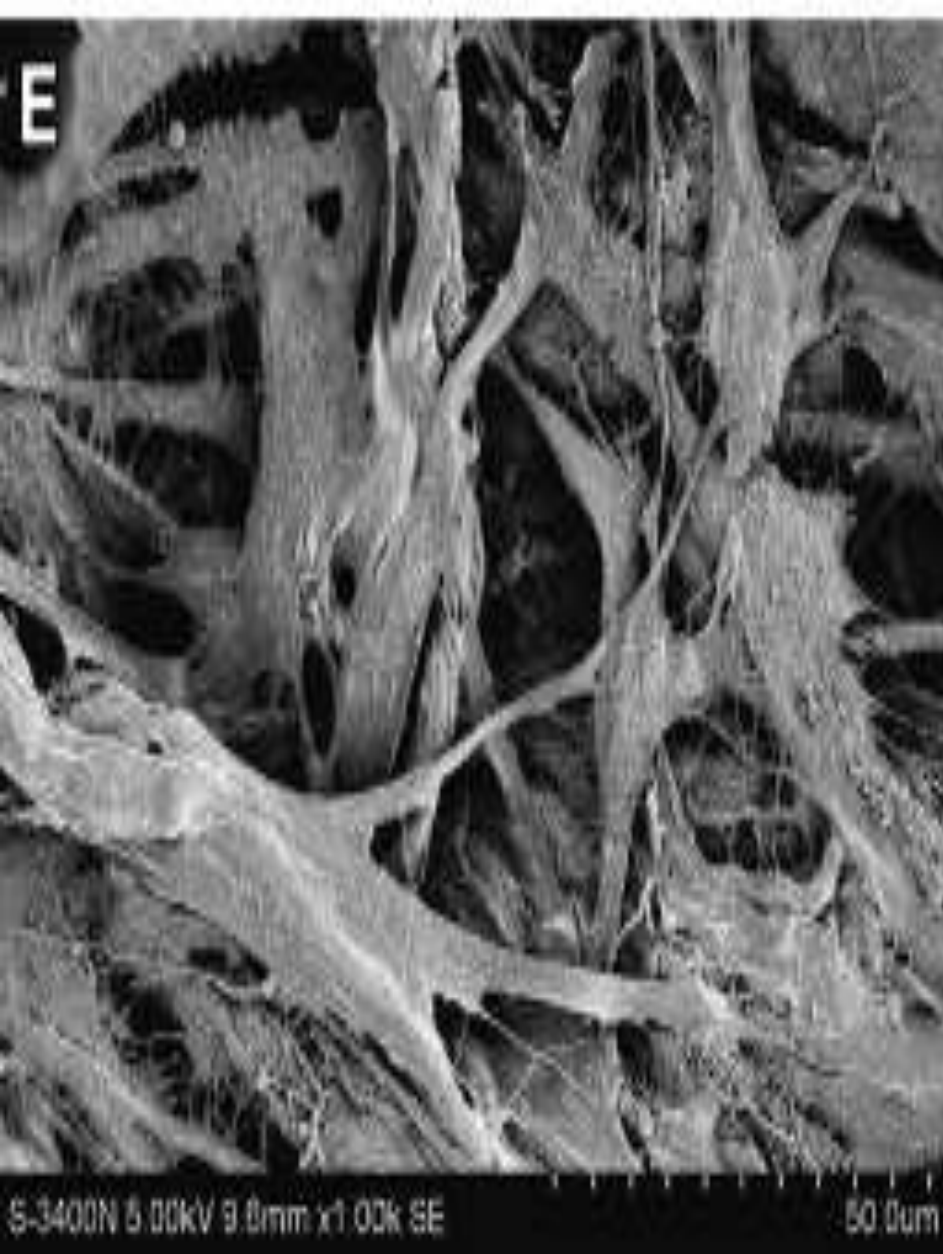
SEM images of macroporous scaffolds fabricated by gas-foaming and salt-leaching process. Uniform interconnectivity and high porosity were observed on both surface (left) and cross-section (right) of scaffolds.



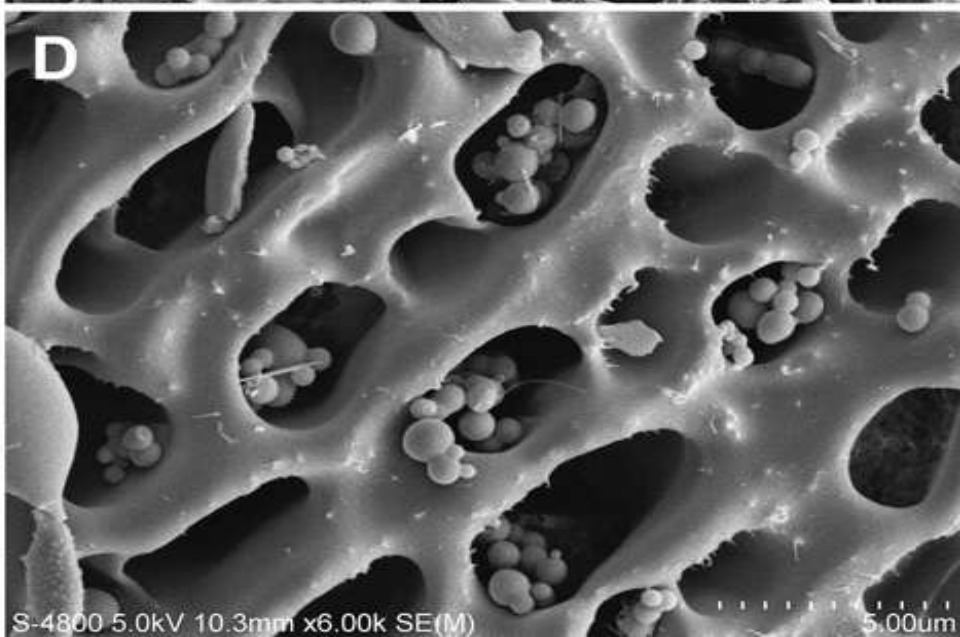
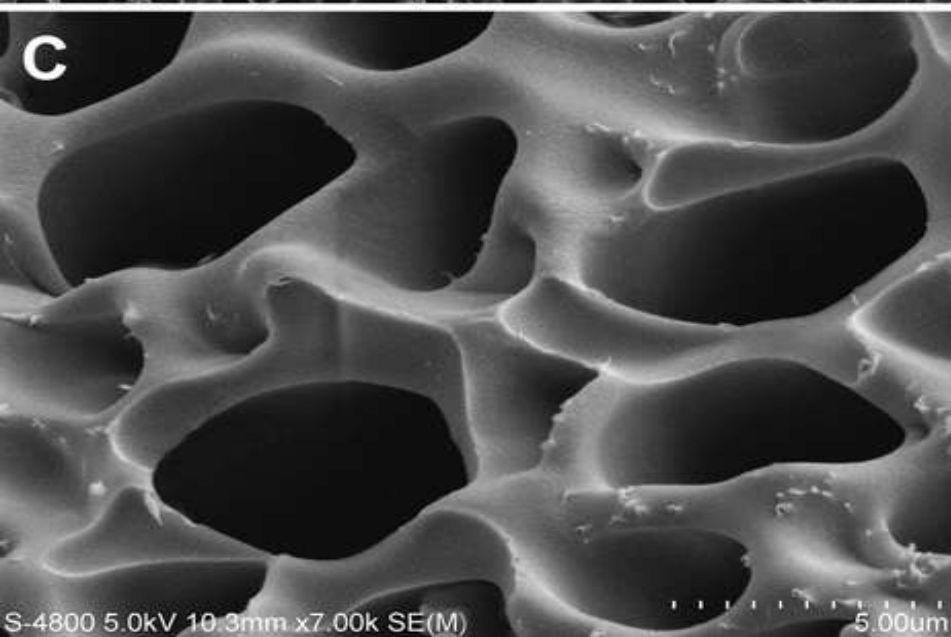
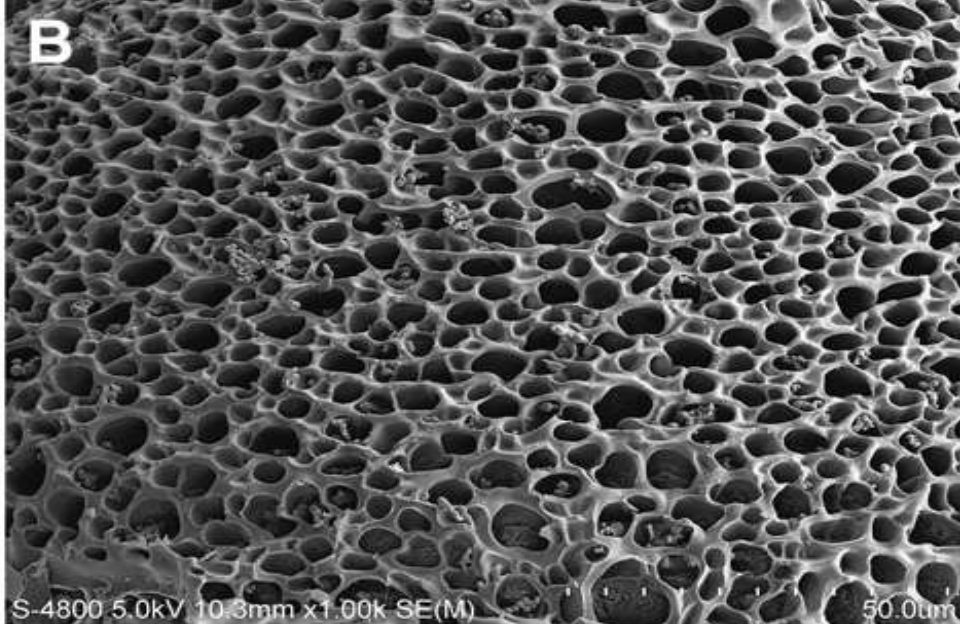
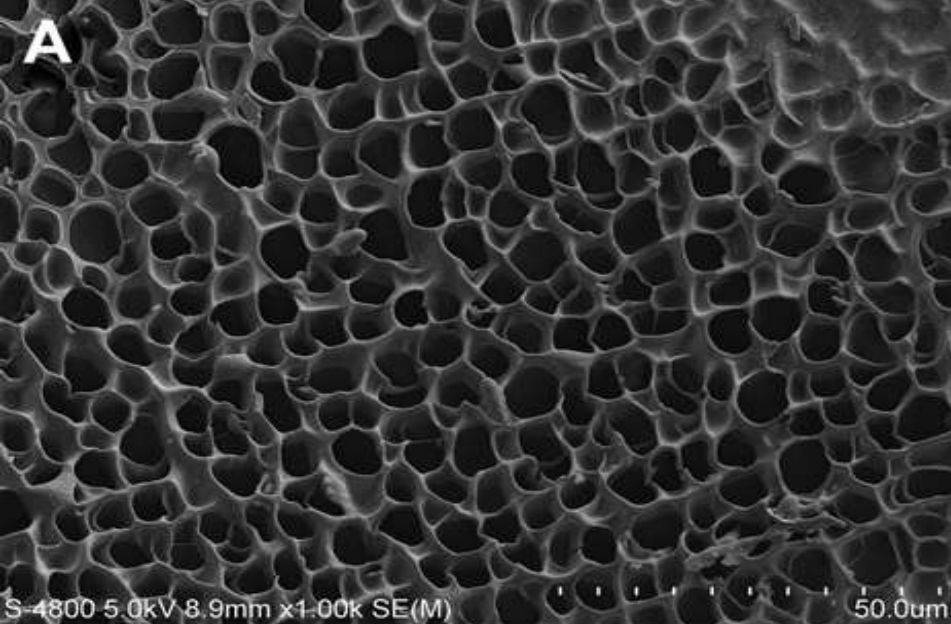
Morphology of a chitosan scaffold obtained by freeze-drying. (A) SEM micrograph; (B) microCT 2D image; and (C,D) respective microCT 3D images



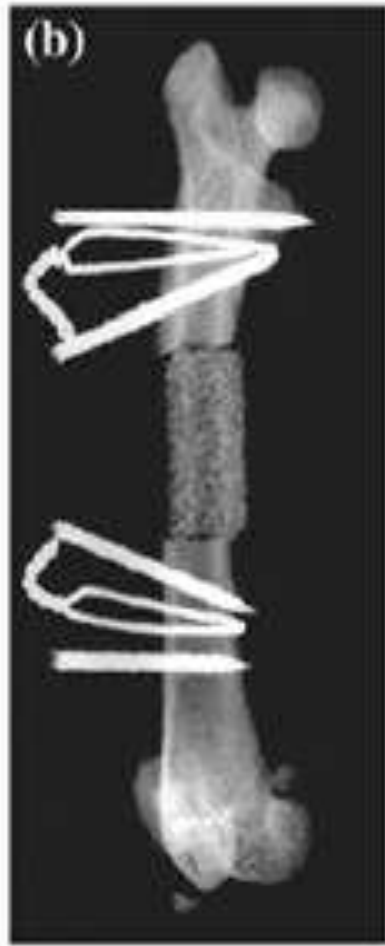
Selected scanning electron microscopic images of collagen membranes seeded with periodontal ligament stem cells. (A) Before cell seeding; (B) Cells seeded on the surface of membrane after 8 h culture; (C) Cells seeded on the surface of membrane after 24 h culture; (D) Cells seeded on the surface of membrane after 48 h culture



(E) Cells seeded on the surface of membrane after 72 h culture; (F) Cells seeded on the surface of membrane after 96 h culture



Selected scanning electron microscopic images of freeze-dried glycidyl methacrylated dextran (Dex-GMA)/gelatin and scaffolds without (A) or with drug delivery systems (B), developed for dual delivery of cells and growth factors to the periodontium. (C) is a regionally magnified view of (A), while (D) is a regionally magnified view of (B)



Radiographs of rat femoral defects (a) prior to implantation, (b) immediately post-implantation, (c) treated with HA/TCP loaded human Mesenchymal stem cells at 12 weeks after implantation (d) treated with HA/TCP alone at 12 weeks after implantation.

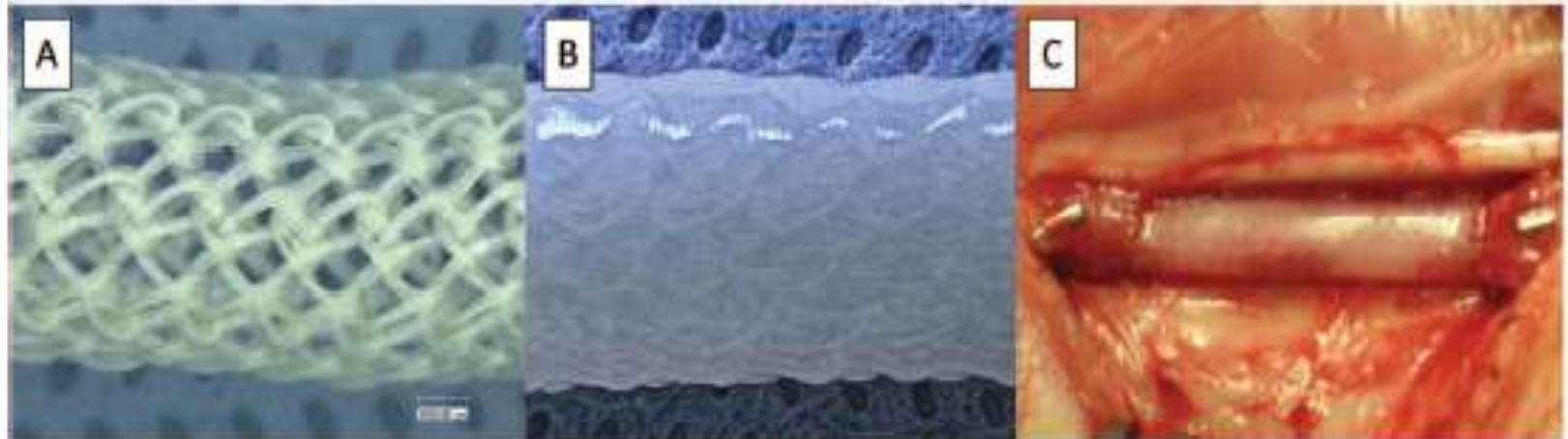
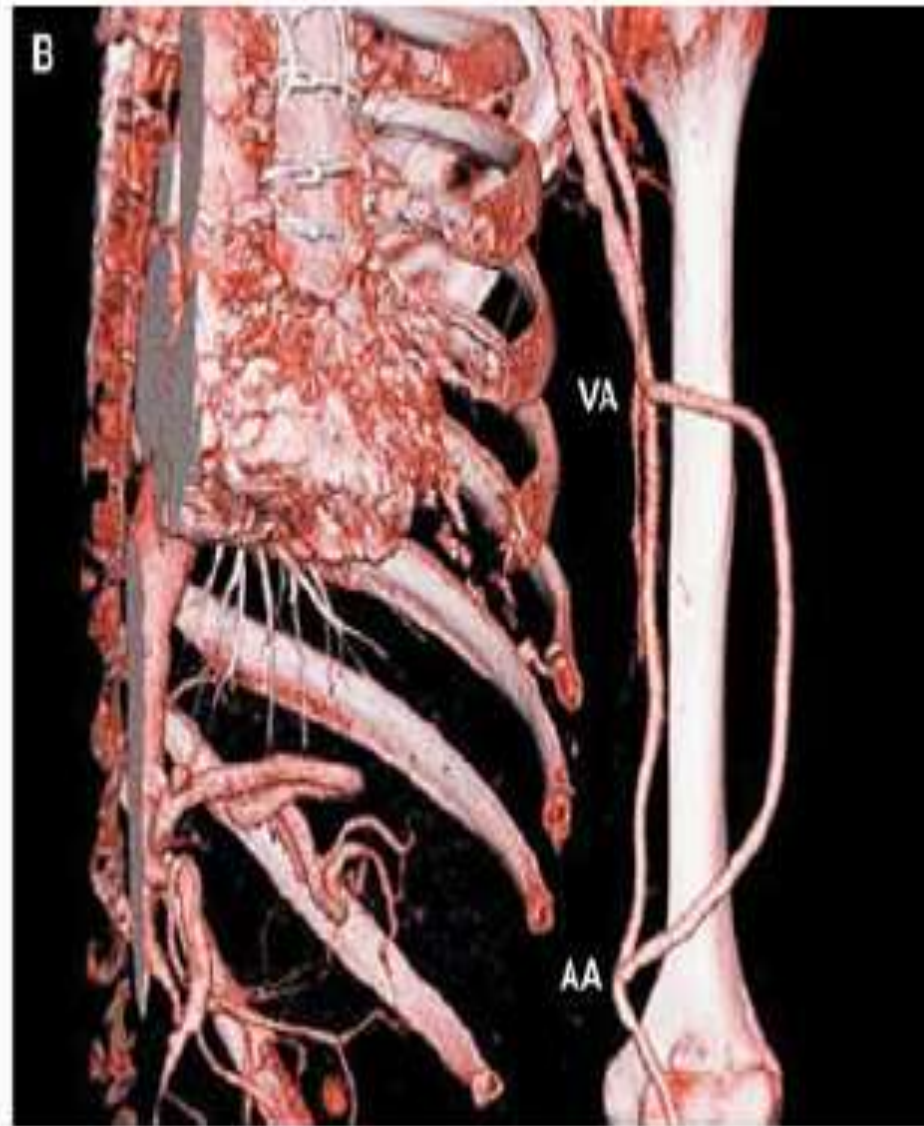
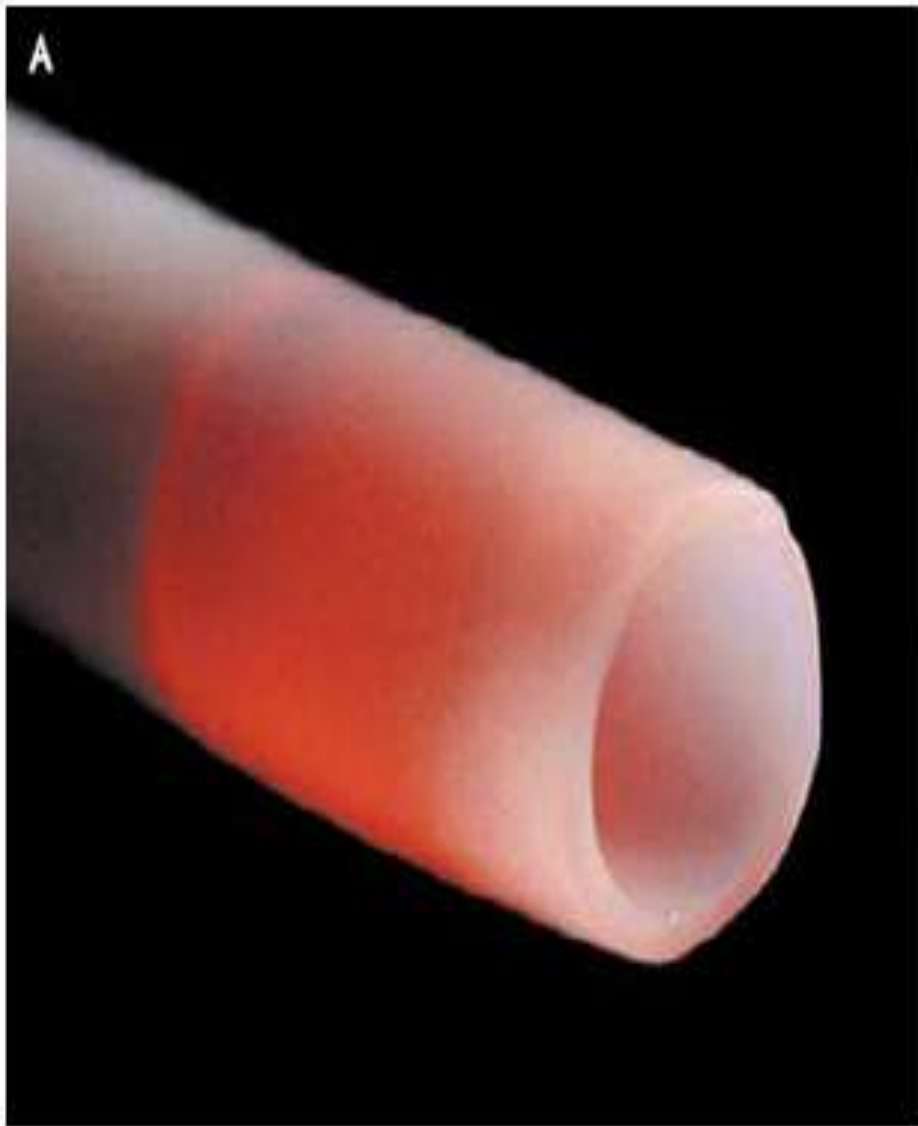
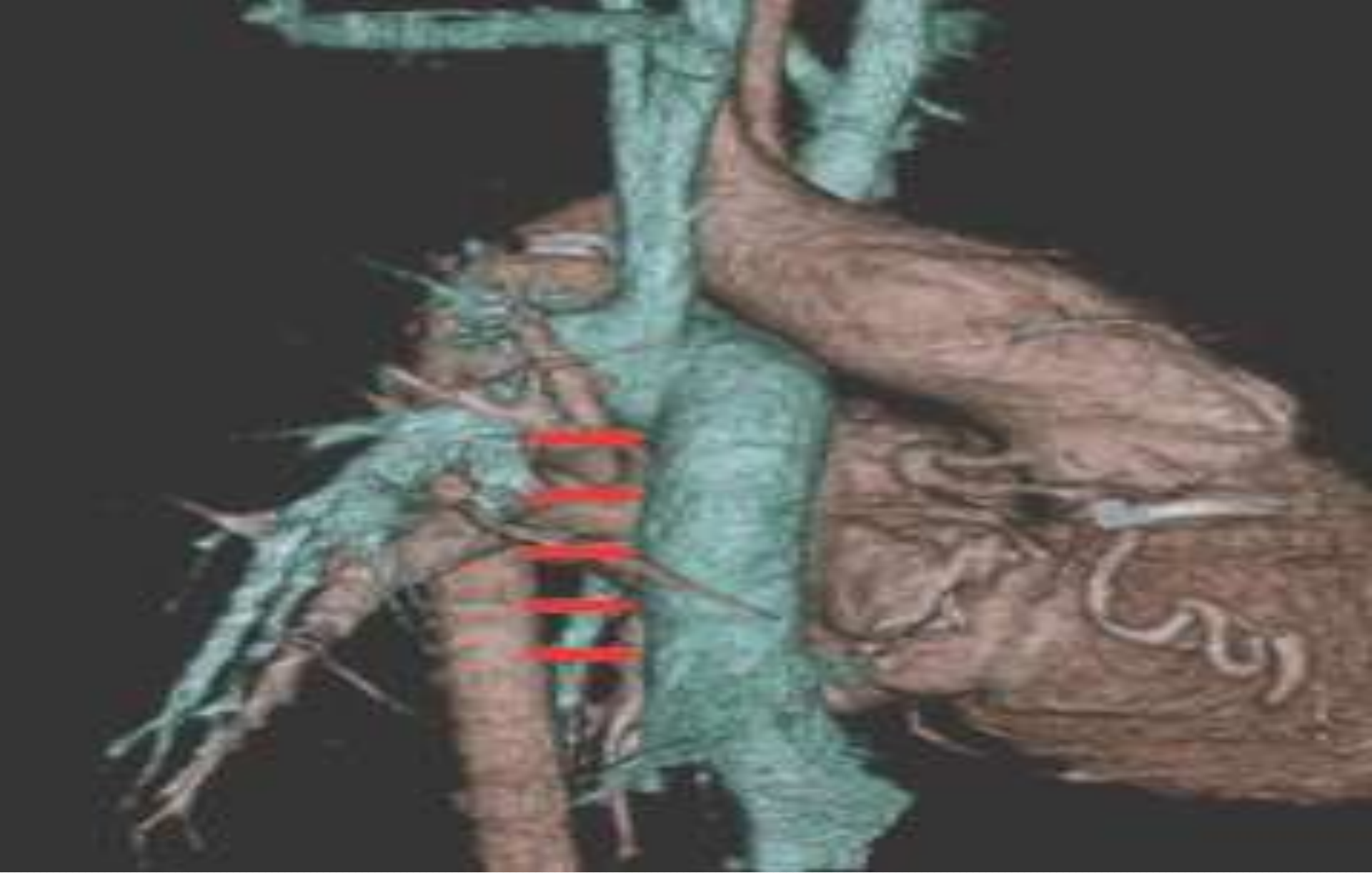


Fig. 7. Bioresorbable, macroporous mesh with a pore size of  $\sim 1\text{mm}$  before [A] and after [B] the embedding into the fibrin/cell matrix. [C] Fibrin-based vascular graft after implantation in the arterial circulation (ovine carotid model)



**The tissue-engineered blood vessel preoperatively (A), at three Months after implantation (B, computed tomographic angiography). VA: venous anastomosis, AA: arterial anastomosis.**



**Three-dimensional computed tomography reconstruction of *in vivo* TEBV. Tissue** engineered vascular graft in a 13-year-old with single ventricle physiology. The graft connects the superior vena cava to the pulmonary arteries. Three-dimensional reconstructed computed tomography of the heart, great vessels, and tissue engineered vascular graft demonstrate a widely patent graft with no evidence of stenosis, thrombosis, or aneurismal dilation

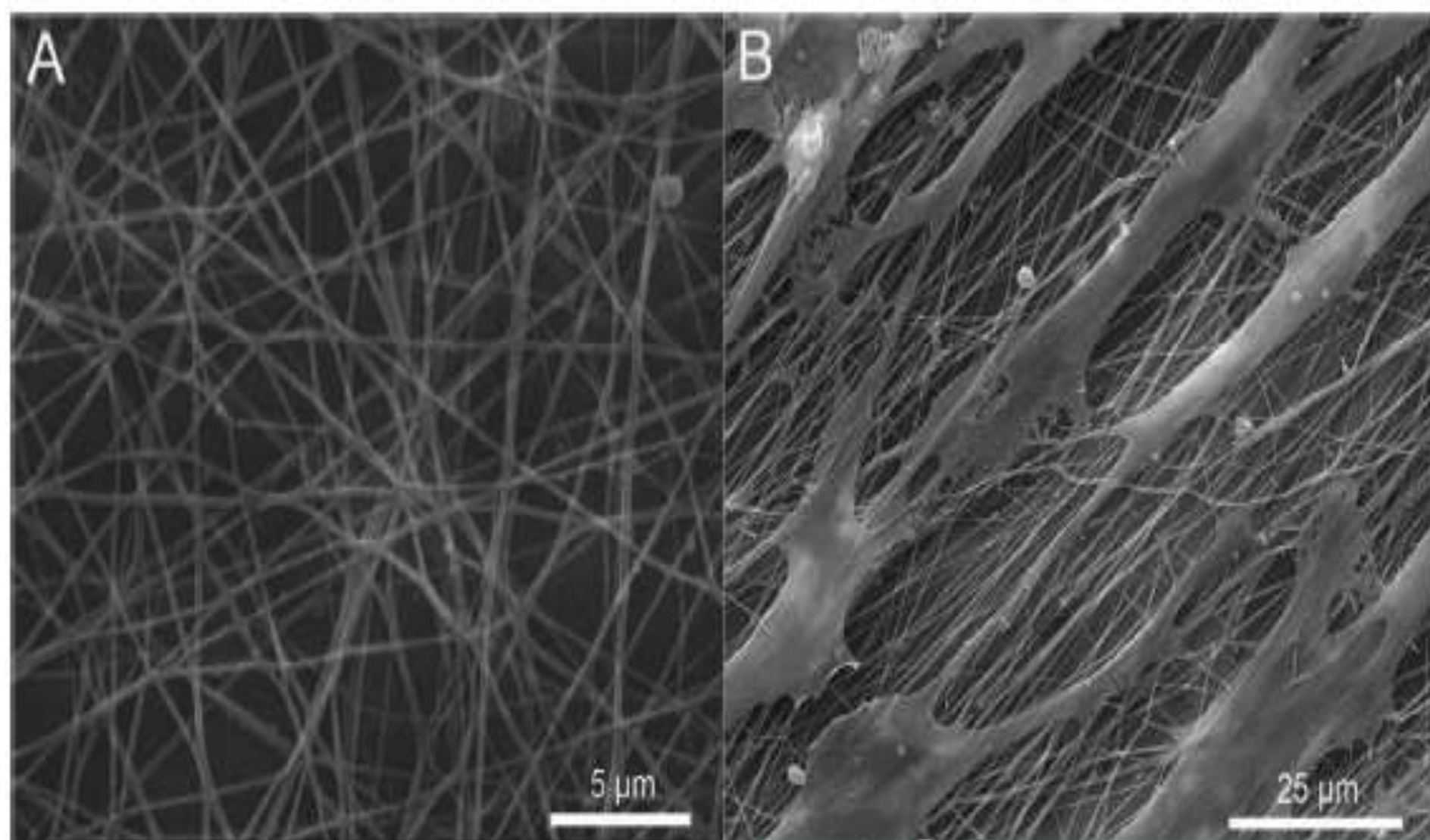


Fig. 3. Scanning Electron Microscopy (SEM) of electrospun PCL-collagen blend nanofibers. A: Randomly spun nanofibers. Magnification 10000 $\times$ . B: Muscle precursor cells cultured on electrospun nanofibers with parallel alignment. The cell growth along the fibers' direction is clearly visible. Magnification 2500 $\times$



Fig. 2. Tissue engineered ACL substitute

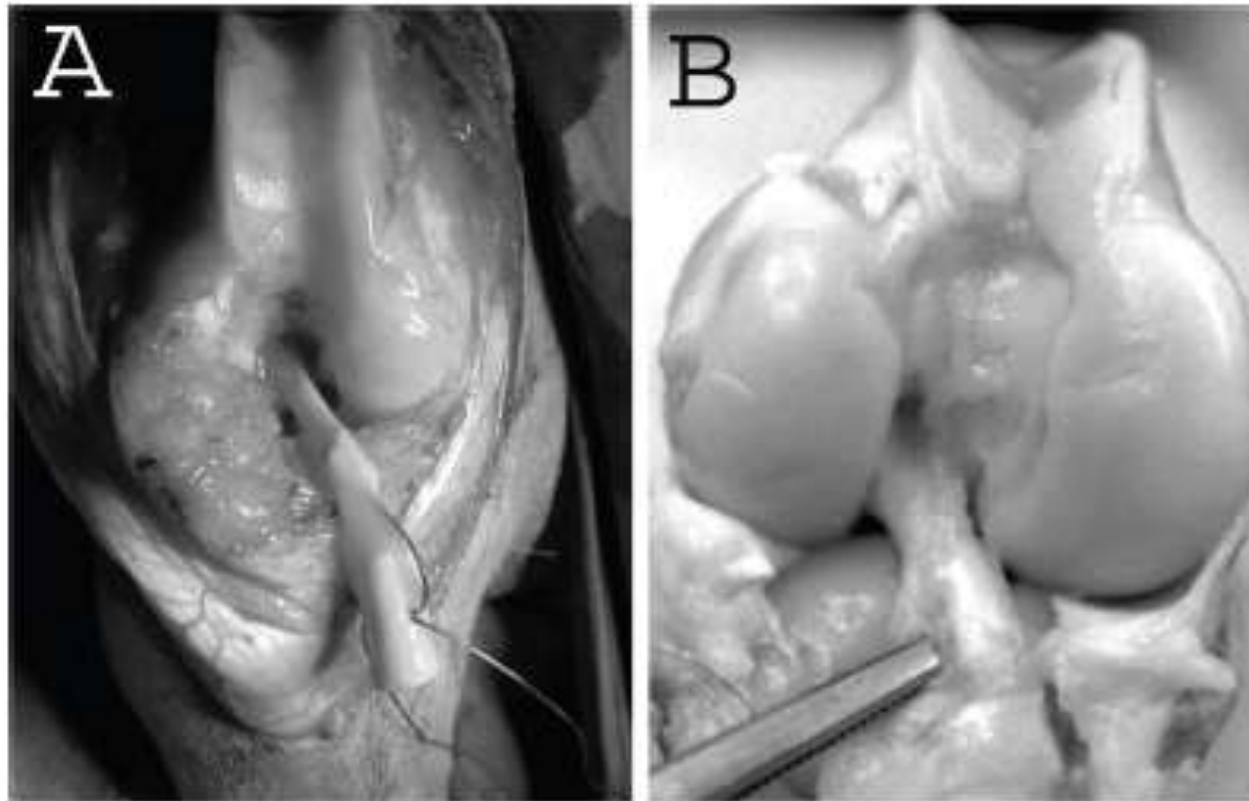


Fig. 5. Implantation of a tissue engineered ACL substitute in a goat knee joint. Macroscopic view of a tissue-engineered ACL substitute at the time of implantation (A) and six months later (B). The articular cartilage didn't show any sign of degeneration before and after tissue-engineered ACL implantation

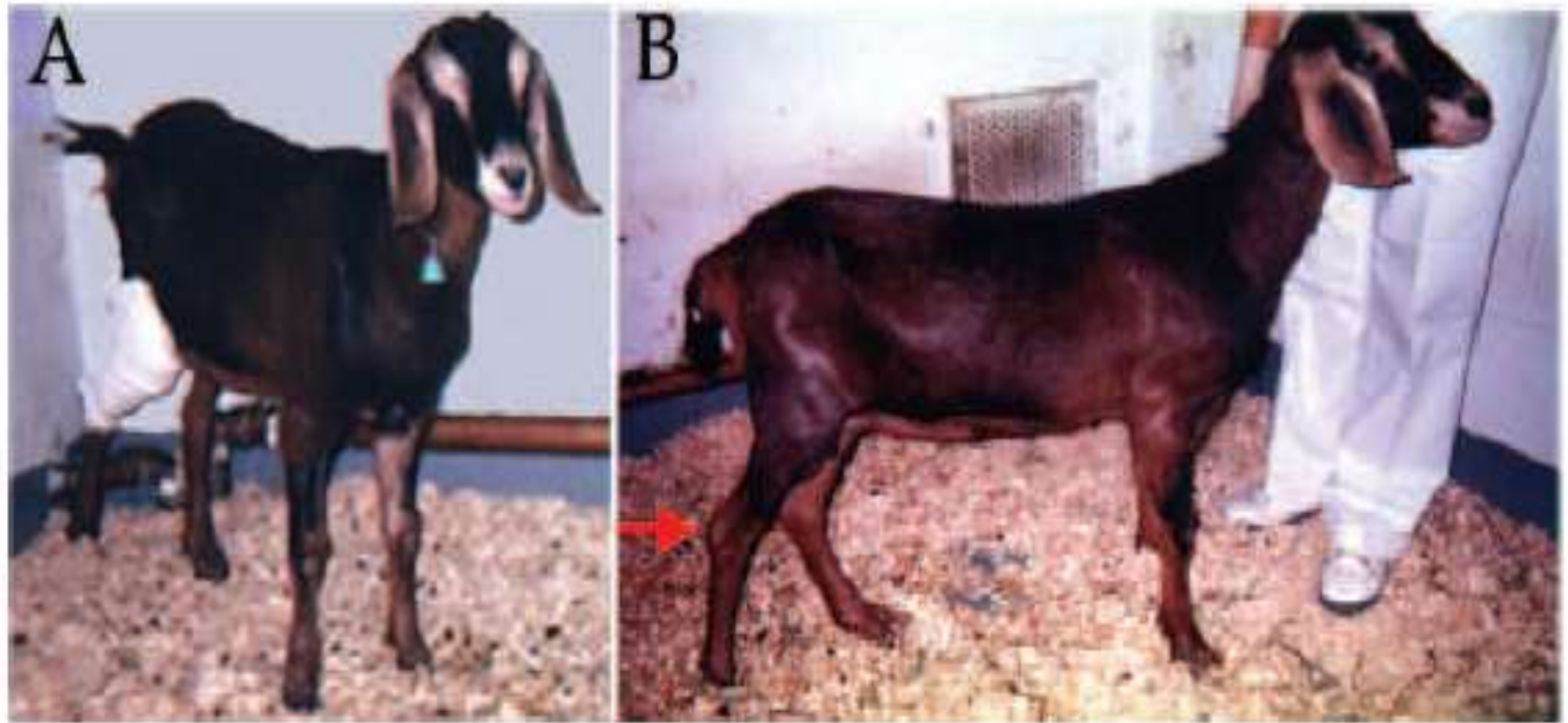
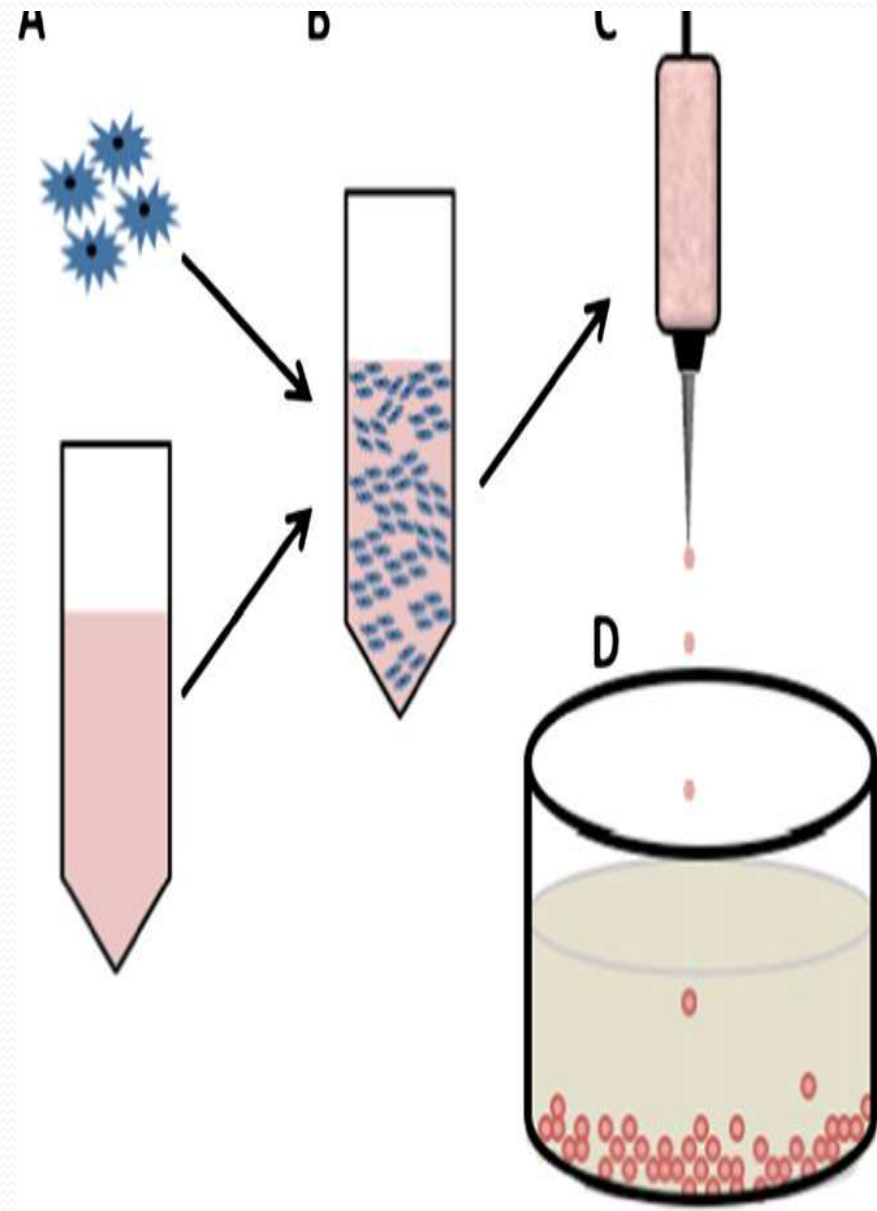


Fig. 4. A goat grafted with an autologous tissue-engineered ACL. Photograph of a goat three days (A) and one month (B) after the implantation in the right knee joint of a tissue-engineered ACL seeded with autologous fibroblasts. The animal could jump and run only one month post-implantation

Schematic of the process of cell microencapsulation with sol-gel transition of hydrogels. (A) In this process, cultured or isolated cells are dissolved in a solution-phase hydrogel (e.g. alginate) and (B) mixed into a viscous cell suspension. (C) The solution-phase hydrogel/cell mixture is then extruded (e.g. by pressure) from a droplet microencapsulator (typically a syringe, possibly with some electric field applied to regulate droplet size). (D) The droplets are collected in a solution promoting gelation (e.g. calcium-containing solution for alginate). Regulation of drop size allows single or multiple cell encapsulation and mechanical properties can be regulated through the hydrogel precursor concentration or the gelling agent



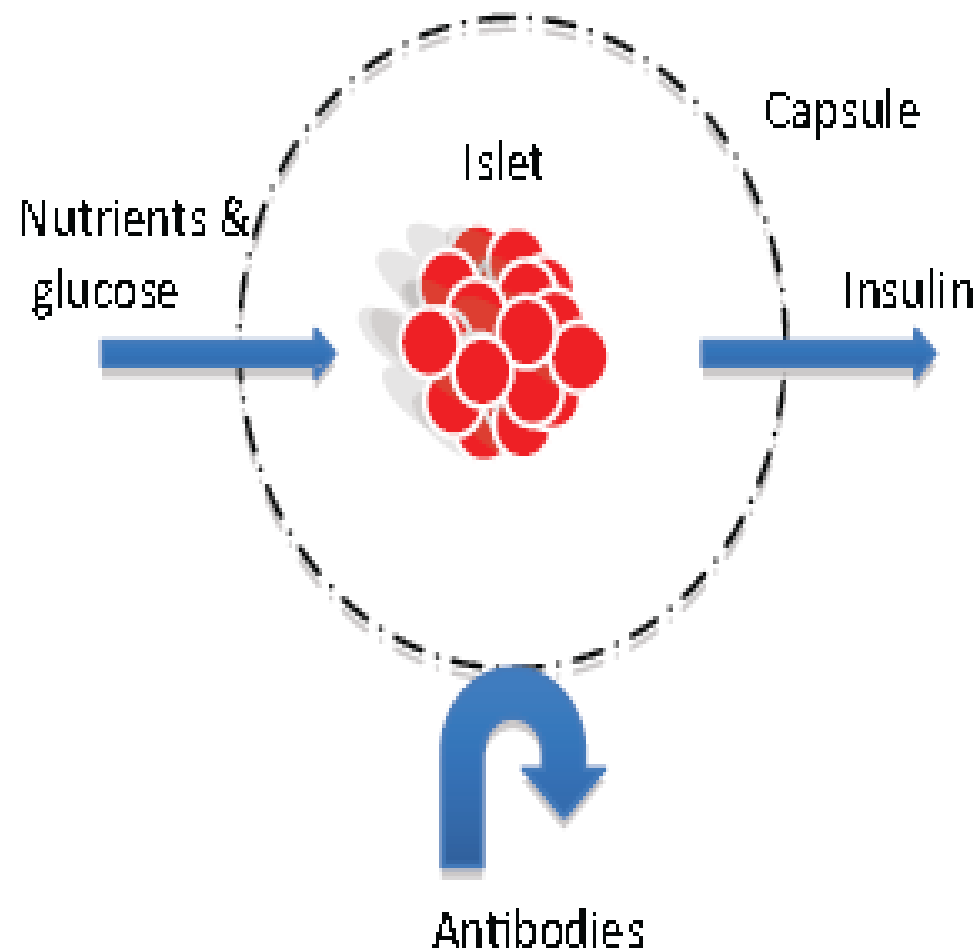


Fig. 3. The concept of microencapsulation of an islet. Nutrients, glucose and insulin can pass through the capsule, but antibodies cannot enter into the capsule



# *Thanks*

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